Handbook of Food and Beverage Fermentation Technology

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MARCEL DEKKER, INC. NEW YORK • BASEL

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

ISBN: 0-8247-4780-1

This book is printed on acid-free paper.

Headquarters
Marcel Dekker, Inc.
270 Madison Avenue, New York, NY 10016, U.S.A.
tel: 212-696-9000; fax: 212-685-4540

Distribution and Customer Service
Marcel Dekker, Inc.
Cimarron Road, Monticello, New York 12701, U.S.A.
tel: 800-228-1160; fax: 845-796-1772

Eastern Hemisphere Distribution
Marcel Dekker AG
Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
tel: 41-61-260-6300; fax: 41-61-260-6333

World Wide Web
http://www.dekker.com

The publisher offers discounts on this book when ordered in bulk quantities. For more information, write to Special Sales/Professional Marketing at the headquarters address above.

Current printing (last digit):

10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

Fermented food is a very interesting category of food products. In every ethnic group in the world, there are fermented foods produced from recipes handed down from generation to generation. Such food products play an important role in cultural identity, local economy, and gastronomical delight. The manufacture of some of the more popular fermented food products has been industrialized, while others are still produced at home using traditional methods with little scientific input.

Fermentation changes the initial characteristics of a food into a product that is significantly different but highly acceptable by consumers. Of course, consumer preference for fermented food varies within and between cultures. For example, within the United States, many consumers like pickles although some do not. The trend in North America is toward acceptance and preference of foreign fermented food products. You can find fermented black beans and black bean sauce (Chinese), kimchi (Korean), and jalapeño peppers (Mexican) in almost every major grocery chain in North America.

Although reference books on fermented foods have been in existence for at least 50 years, those with details on the science, technology, and engineering of food fermentation began to appear after 1980. Scientific literature in the past decade has been flooded with new applications of genetic engineering in the fermentation of food products, especially in the dairy field.

This book provides an up-to-date reference for fermented foods and beverages. Almost every book on food fermentation has something not found in others. The *Handbook of Food and Beverage Fermentation Technology* provides a detailed background of history, microorganisms, quality assurance, and the manufacture of general fermented food products, and discusses the production of seven categories of fermented foods and beverages:

Semisolid dairy products, e.g., sour cream
Solid dairy products, e.g., cheese
Meat products, e.g., sausages
Soy products, e.g., soy sauce
Vegetables, e.g., Korean kimchi
Cereal foods, e.g., sourdoughs
Beverages, e.g., fermented milks

Traditional fermented products are discussed, including yogurt, cheese, sausages, tofu, sauerkraut, sourdoughs, and whiskey. We also present details of the manufacture and quality characteristics of some fermented foods that may not be included in other books in the English language. These include fromage frais, Scandinavian cheeses, fungal sausages, miso, Chinese pickles, African kenkey, and semifermented tea. Although this book has several unique characteristics, many topics are omitted for a variety of reasons, including space limitation, product selection, and the contributors' areas of expertise.

This book is unique in several aspects: it is an updated and comprehensive reference source, it contains topics not covered in similar books, and its contributors include experts from government, industry, and academia worldwide. The book has 47 chapters and is divided into eight parts. It is the cooperative effort of 59 international contributors from 17 countries with expertise in one or more fermented products, led by an editorial team of seven members from three countries. In sum, the approach for this book makes it an essential reference on food fermentation.

The editorial team thanks all the contributors for sharing their experience in their fields of expertise. They are the people who made this book possible. We hope you enjoy and benefit from the fruits of their labor.

We know how hard it is to develop the content of a book. However, we believe that the production of a professional book of this nature is even more difficult. We thank the production team at Marcel Dekker, Inc., and express our appreciation to Ms. Theresa Stockton, coordinator of the entire project.

You are the best judge of the quality of this book.

Y. H. Hui
Lisbeth Meunier-Goddik
Åse Solvejg Hansen
Jytte Josephsen
Wai-Kit Nip
Peggy S. Stanfield
Fidel Toldrá

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Origin and History of Food Fermentations

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If you know the history of man's food, you know the history of man.

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I. INTRODUCTION

According to current scientific thought, the universe is approximately 15 billion years old and the Earth is approximately 4.5 billion years old. Fossil microorganisms have been found in rocks 3.3 to 3.5 billion years old (1). They were the first forms of life to appear on Earth. They were likely the blue-green algae, which contain a pigment enabling them to use the sun's radiation to synthesize carbohydrates. They contain deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) similar to all other forms of life today. They also contain the enzymes, proteases, amylases, lipases, and other enzymes required to hydrolyze proteins, starches, and lipids necessary for recycling. This was very fortunate because microorganisms have been required ever since as recyclers of organic matter. Without them, the Earth would be a giant dumping ground containing all forms of organic matter and dead bodies. Initially, microorganisms consumed organic matter, including dead organisms, as food for their own energy requirements. Later, after plants and animals evolved, microorganisms consumed dead plants and animals and became able to invade plants and animals, causing disease—in some ways the first stage in recycling.

The next forms of life, evolving about a billion years later, were plants, also based upon DNA/RNA and having the ability to convert carbon dioxide and water to carbohydrates using the sun's radiation for energy. The plants became the food supply for future evolution of animals, including humans. Microorganisms recycled the plants, consuming them as food/energy as they died, returning them to soil for future plant growth. Plant life evolved in a sea of microorganisms and thus had to have means of resisting invasion (plant disease) even when alive. They did this by developing a ligno-cellulose structure that resists microbial invasion. A seed germinating in the soil has to survive the onslaught of billions of microorganisms that, given the opportunity, would destroy the seed and/or recycle the developing plant. Plants, their leaves and roots—cassava, sweet potatoes, yams—berries,

fruits, nuts and cereal grains—particularly wheat, rice, maize, barley, rye, oats, millet, and sorghum—and legumes, beans, and peas are major staples of our food supply today.

Millions of years before humans appeared on earth, all the chemical and enzyme reactions needed for food fermentations were present as part of the recycling reactions used by microorganisms to digest and recycle plant components; for example, fermentation of fruits and fruit juices to wine and vinegar, germination of grains as the first step in brewing alcoholic beverages, and souring of milk. When humans and other animals evolved on Earth, they had to consume the food supply either before it was invaded by microorganisms and recycled or while in various stages of recycling—the fermented foods. When microbes produced unpleasant aromas or flavors in the food or produced toxins that caused illness or death, the food was spoiled and humans learned to avoid it. If the invasion of the food components by microorganisms yielded attractive aromas, flavors, and textures, humans learned to appreciate and desire such foods. These were the beginning of fermented foods, including sour milk, cheeses, wines and beers, vinegar, lactic acid products such as sauerkraut, and hundreds of other fermented foods consumed today.

There is another factor related to fermented foods and lost in antiquity, and that is salt: common salt, sodium chloride, or sea salt—a mixture of sodium, potassium, magnesium, and calcium salts found in seawater. Salt has been highly prized for thousands of years. In ancient times, soldiers received part of their pay in the form of salt (or salary) and, even today salt is vital to producing savory foods, based primarily on its condiment value, but it was also valued throughout history as a preservative. Salt in suitable concentrations, prevents putrefaction and leads to a controlled protein hydrolysis.

When ponds of seawater dry up under the influence of temperature and wind-flow (actually a method of producing sea salt even today), the seawater may contain fish and other sea animals that isolated in the seawater die their bodies are self-autolyzed by their enzymes, leading to amino acid/peptide concentrations. It is likely that humans discovered that such animal residues in high salt brines were savory condiments.

Humans also discovered very early that salt could preserve fish or other animal tissues, especially when they were sun-dried, and such salted, sun-dried fish are staple foods in many marine areas of the world today.

Seawater also may have played a role in primitive lactic acid fermentation/preservation of plant materials because such materials stored in seawater would likely undergo lactic acid fermentation as well.

It has been hypothesized by anthropologists (2) that it was alcoholic fermentation and the desire for alcohol that motivated humans to settle down and become agriculturists. Humans could not have survived over the millennia without fermented foods. Fermentations preserve foods, improve digestibility and enrich substrates with essential vitamins, amino acids, and fatty acids. They also convert vegetable proteins to savory meat-like flavors and textures and yield the diverse flavors and aromas that enriched the human diet in the past, enrich our diets today, and will continue to do so in the future.

II. HISTORY OF SELECTED FERMENTED FOODS

A. Alcoholic Fermentations

Mead/Honeywine. Honeybees have been producing honey from flowering plants and probably also from honeydew for 10 to 20 million years before humans appeared on earth (3). Honey was the world's first concentrated sweet. Its sugar concentration (about 80%) is too high for honey to undergo fermentation or even spoilage without dilution. It was the reserve food for the honeybees themselves but also sought after by humans and animals such

as bears. Diluted with water such as rain, however, it will undergo fermentation by yeasts that live in the surrounding environment. So it is likely that honey/mead fermentation was occurring long before humans arrived and continues as a fermentation today.

Primitive wines and beers are vastly different from our modern wines and beers. The former are generally cloudy, effervescent beverages containing not only liquid but particles of the fermenting substrate, yeast cells along with the alcohol, and B vitamins. They are very nutritious and energy-rich.

An example is African kafir/sorghum beer. The art of kafir beer production goes back to prehistoric times. In the villages, kafir beer is made by women: girls learn how to make kafir beer for their husbands before they marry (4). Sorghum grains or millet are germinated, sundried, ground, and mixed with sorghum, millet, or maize flours and water, and then cooked, cooled, and fermented by the residual yeasts and the dregs in the containers. Fermentation is carried out in large crocks or drums (5).

Fermentations involving production of ethanol are among the most ancient fermentations known. The most primitive methodology utilizes chewing the grains to introduce saliva (ptyalin) as a source of amylase to hydrolyze the starch to sugar and has been used for centuries. An example is chicha, produced in the Andes region of South America (6). Even today, women and children sit in a circle chewing maize kernels. The gobs are then removed from the mouth and sundried. Later they are placed in crocks covered with water and allowed to ferment with yeasts in the environment. The yellow colored cloudy liquid contains as much as 6% ethanol and a wide variety of B vitamins. In ancient Incan times, the emperor himself could hold office only as long as he delivered sufficient chicha to the citizens. In ancient Japan, rice wine/sake was also produced using chewing of grains as a source of amylase to convert the starch to sugar (7). Later it was discovered that rice overgrown with *Aspergillus*, *Rhizopus*, or *Mucor* molds also became sweet and could be fermented to rice wine. Among the more complex sweet/sour alcoholic foods are tapay, tapai, tape' and Chinese Lao-chao. These generally rely on two or more fungi for their production. These can include *Amylomyces rouxii*, a yeast-like mold, and *Endomycopsis fibuliger*, a mold-like yeast (8).

Thousands of years ago in Egypt, wheat grains/flour were made into lightly baked bread that was then moistened with water and fermented to a primitive beer—bouza.

Still earlier in human history, at the dawn of agriculture, when grains were collected in crocks, it is highly likely that such grains, on occasion, became moistened with rain, germinated, and fermented to primitive beers.

The most ancient Mexican alcoholic beverage is *pulque*, made by fermenting pulp juices from the *Agave* plant. *Leuconostoc mesenteroides* produces dextrans that add texture to the beverage. The alcohol is produced by *Saccharomyces cerevisiae*, a yeast, or by *Zymomonas mobilis*, an alcohol-producing bacterium. Pulque is very rich in B vitamins and plays a vital role in the nutrition of, in particular, the economically disadvantaged in Mexico (4).

B. Vinegar—the Acetic Acid Fermentation

Primitive alcoholic beverages generally contain some acetic acid, but the amount is limited as long as the fermentation remains anaerobic. The rapid production of carbon dioxide helps maintain anaerobiosis by providing a layer of CO_2 on the surface of the fermenting materials. However, when the alcoholic fermentation stops, *Acetobacter* sp. become active as soon as oxygen becomes available, and a portion of the ethanol is converted to acetic acid—vinegar. Vinegar is an ancient condiment and extremely useful as a pickling agent or even as a medicinal because it is germicidal.

Savory/Meat Flavored Sauces and Pastes. It is not known who discovered how to transform bland vegetable protein into meat-flavored sauces and pastes. It may have been an accident; nevertheless, it was one of the great discoveries in food science. When seeds fall upon the ground, they either germinate, forming new plants, or they become overgrown with microorganisms as the first step in recycling. The seed coat is rather resistant to microbial growth, so the first organisms to penetrate into the cotyledons are often molds that produce proteases, lipases, and amylases that hydrolyze the various components in the seed. Thus, the mold–overgrown seeds become a source of enzymes. Of scientific importance, such moldy seeds are described as a "koji" and can be used to hydrolyze the proteins, lipids, and starches in other vegetable or animal products. The first koji in recorded literature was a millet kogi. Millet koji was mixed with meat, fish, or fowl and salt and stored in a bottle for 100 days. The first reference to meat-flavored pastes was made about 3000 years ago during the Chou dynasty in China (9). The first reference to soybeans as a substitute for meat was in the world's oldest encyclopedia of agriculture, published in A.D. 535 in China.

Soybeans, rich in protein, are an excellent source of nutrition. In order to be palatable, they must be hydrated/soaked and cooked. As long as soybeans remain dry they are not susceptible to microbial spoilage. After being hydrated, however, they become susceptible to overgrowth by bacteria and molds, as is true of most seeds. The first savory products were all mashes or pastes. It was not until about A.D. 25–220. that liquid sauces appeared in the literature in the Han dynasty (9).

In a simple primitive process of producing savory soybean paste, soybeans are soaked and cooked and made into a ball covered with rice straw and placed under the ceiling of the house where it is warm. *Aspergillus* molds present in the straw overgrow the soybeans in approximately 30 days. The mold-covered soybeans are then mixed with sea salt brine and allowed to digest for a year or longer. Enzymes from the mold digest the proteins, lipids, and carbohydrates, yielding savory amino acid/peptide–flavored soybean paste. Liquid released from the soybean paste is a tamari-type soy sauce very rich in savory amino acid/peptide flavors (10).

We can only guess what effect soybean paste and soy sauce had on consumers used to eating predominately bland rice. It was one of the great discoveries of food science, and along with soy sauce and miso we have Nestlè "Maggi"-type meat flavors and bouillon cubes in today's markets.

C. Fermentations Yielding Meat-like Textures

Indonesian tempeh fermentation is closely related to soy sauce fermentation as the first stage is an overgrowth of soybeans with a mold, *Rhizopus oligosporous* or related strains (4,11,12). The fungal mycelium knits the soybean cotyledons into a compact cake that can be sliced thin and deep-fat fried or cut into cubes for use in soups. This fermentation has been carried out in Indonesia for hundreds of years by people untrained in microbiology or chemistry—yet they have the ability to produce high-quality tempeh.

The most surprising thing about tempeh fermentation is that in recent years a new high-technology industry has developed with the objective of producing meat substitutes (4,13–15). There are two major methods. The first is to extract soybean protein and spin it into fibers by passing the protein strands through a chemical bath. The resulting fibers are oriented to a meat-like texture and meat flavors are added. The dehydrated chunks are used in soups and other food products as vegetarian meat substitutes. It is a very sophisticated and relatively expensive food processing technique. Indonesian tempeh achieves much the same objective by fermentation in which mold mycelium provides the

meat-like texture and the resulting products are within the financial means of the average Indonesian.

A second method of producing meat substitutes is even more closely related to the tempeh process in that it involves growing edible strains of *Fusarium graminearum* mold mycelium, harvesting the mycelium by centrifugation/filtration and adding meat flavors, and then dehydration. This process was developed by Rank, Hovis, MacDoughall (RHM) in England (16,17). The nuggets are based on mold mycelium for texture plus added flavors. This technology is advanced and sophisticated and relatively expensive compared to the tempeh process.

Indonesian tempeh achieves a similar degree of texture as a meat substitute by overgrowing soaked, dehulled, cooked soybean cotyledons with *Rhizopus oligosporous* mycelium.

D. Lactic Acid Fermentations

Lactic acid fermentations are among the most ancient and important fermentations in the world: they enabled the human race to survive and thrive and they remain very important in the diets of humans today (4).

Lactic acid fermentations became known to humans as soon as they started domesticating and milking cows, sheep, and goats. People had to store the milk in a container, and one of the earliest containers was the stomachs of slaughtered animals. Milk sours very rapidly because of its natural content of lactic acid bacteria. Sour milk became one of the first fermented foods after humans settled down and became agriculturists, and it lives on in the form of yogurts today. Stored in animal stomachs, the sour milk curdled, lost its whey, and became primitive cheeses through the activities of other lactic microorganisms in the environment. For millennia, cheeses have been an important part of the diet of humans and they remain so today.

Lactic acid fermentations are very energy efficient, generally requiring no heating or cooking either before or after fermentation. A prime example of lactic acid vegetable fermentations is the sauerkraut fermentation. Fresh cabbage is shredded and mixed with 2.25% w/w salt (sodium chloride). The salted cabbage is placed in a crock and covered with a lid or a plastic cover that allows no penetration of air. The natural fermentation (no inoculum required) begins with the development of *Leuconostoc mesenteroides*. *L. mesenteroides* produces both lactic acid and carbon dioxide, which flushes out any residual oxygen, helping to maintain anaerobic conditions. The second organism that develops is *Lactobacillus brevis*, which produces additional lactic acid and carbon dioxide. This is followed by *Lactobacillus plantarum,* which produces additional acid. The last organism to develop is *Pediococcus cerevisiae,* which produces additional acid. The final product has an acidity of about 1.7% to 2.3% acid (as lactic) and has excellent keeping quality as long as the product is kept anaerobic. The sauerkraut can be eaten fresh without cooking as a salad or cooked as a hot food.

Another lactic acid fermentation is that of Korean kimchi, which is a staple in the diet of the average Korean, who may eat 100 g a day in summer and 150 g a day in the winter. In Korean kimchi, Chinese cabbage is a prime substrate but radishes, red peppers, and other vegetables may be included. The vegetables are shredded and immersed in a strong salt brine (5–7% salt for 12 hours or 15% salt for 3–7 hours) followed by draining and rinsing. The subsequent fermentation time depends on the temperature of fermentation (one day at 30°C or 3–60 days at 5°C). Kimchi is less acidic than sauerkraut and the product is carbonated.

Lactic acid fermentation has been applied to most vegetables such as cucumbers and carrots, and some green fruits, such as limes and olives. It has been utilized by Indian farmers to preserve excess vegetables during the growing season. Lactic acid fermentation is utilized throughout the world as a prime method of preserving fresh vegetables.

It is likely that bread fermentations began as soon as humans started to use fire/cooking and grinding starchy grains such as barley, wheat, millet, rye, and sorghum to make them more easily consumed. Such flours slurried with water, immediately begin to ferment by lactic acid organisms and yeasts in the environment. These microorganisms struggle for survival in the increasingly acidic mixture. The outcome generally includes one or more lactic acid species and one or more yeasts. If the flour-water slurry is dense enough to form a dough or pancake-like structure and it is baked, it will yield, depending on the conditions, leavened or sourdough-like breads. Since at least 5000 B.C., breads have played a significant role in human diets. Wheat flours contain gluten, which retains the carbon dioxide produced by heterofermentative lactic acid bacteria and yeasts fermenting symbiotically.

Rice does not contain gluten, so it cannot yield leavened bread, but people from the region known today as India discovered a way of producing bread-like foods from rice by combining fermentation of rice with that of legumes such as black gram. Both ingredients are soaked in water and then ground in a mortar to make a stiff batter that when incubated overnight is leavened (rises) so that it can be steamed (Indian *idli*) or cooked as a pancake (Indian *dosa*) adding leavened bread–like products to the Indian diet (4).

III. SUMMARY

Fermented foods go back to the origins of microorganisms, the first forms of life on Earth followed by the evolution of plants—the basis of human foods—and the subsequent interrelationships between microorganisms that have the task of recycling organic matter and the plants upon which humans and all animals depend for food and energy. Plants and plant materials (foods) are subject to recycling by microorganisms as soon as grown. If they are harvested and consumed immediately (e.g., fresh fruits and berries), there has been little, if any, fermentation or recycling. Recycling and fermentation are acceptable as long as the products are attractive in flavor and aroma and do not contain any toxic products. If the flavors and aromas are unacceptable to the consumer or the plant materials contain toxic material, the potential foods are described as "spoiled. " Fermented foods are consumed in various stages of recycling. The human race has been dependent on acceptable degrees of recycling and food fermentation from the beginning of history and remains dependent still today, although modern technology—canning, freezing, and dehydration—enables humans to postpone recycling and preserve foods for extended periods of time.

The human race has depended upon fermented foods as major sources of food and energy over millennia, continues to do so today, and will do so for the future.

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Microorganisms

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I. INTRODUCTION

The aim of this chapter is to give an overview of microorganisms used in the production of fermented foods. More than 50 species of microorganisms are frequently used in food production, and therefore this chapter will focus mainly on common aspects of the use of microbial cultures, rather than on the differences between the cultures and species. The chapter is intended as a guide for the practical oriented food engineer on how to apply microorganisms in the production of industrially produced fermented foods.

II. FERMENTED FOODS

A. Traditional Food Fermentations

Food fermentation has been used for centuries as a method to preserve perishable food products. The raw materials traditionally used for fermentation are diverse and include fruits, cereals, honey, vegetables, milk, meat, and fish. Fermented products encompass, but are not limited to wine, beer, vinegar, bread, soy sauce, sauerkraut, kimchi, pickled olives, different fermented milk products, a large number of cheeses, and a variety of sausages. Popular fermented foods are listed in Table 1 together with the raw materials used and the type of culture involved in the fermentation. Fermentation was invented long before the discovery of microorganisms and the mystery of the process is reflected in the common origin of the words for yeast and ghost. It was understood that some processes required an inoculum, and the need for this was satisfied by keeping a sample from the previous production. This procedure is still in use for propagation of sourdough for private use, and also for the production of some artisanal cheeses. For other processes, inoculation was not necessary because naturally occurring microorganisms in the raw materials could, under proper conditions, be a reliable source of the microbial flora. This is the case in the production of raw milk cheeses, wine, sauerkraut, and some fermented sausages. The production of fermented foods and the characteristic qualities of each are described in detail in other chapters of this handbook. Recent comprehensive reviews of fermented foods have been edited by Wood (1).

Table 1 Fermented Foods and the Required Ingredients

Product	Raw material	Starter culture
Beer	Cereals	Yeast
Wine	Grape juice	Yeast, lactic acid bacteria
Vinegar	Wine	Acetic acid bacteria
Bread	Grains	Yeast, lactic acid bacteria
Soy sauce	Soybeans	Mold, lactic acid bacteria
Sauerkraut, kimchi	Cabbage	Lactic acid bacteria
Fermented sausages	Meat	Lactic acid bacteria
Pickled vegetables	Cucumbers, olives a.o.	Lactic acid bacteria
Fermented milks	Milk	Lactic acid bacteria
Cheese	Milk	Lactic acid bacteria, yeast, mold

B. Industrial Food Fermentations

With the discovery of microorganisms, it became possible to understand and manage food fermentations. Methods for isolating and purifying microbial cultures became available in the 19th century. Sterilization or pasteurization of the raw materials prior to inoculation with well-defined cultures allowed the fermentation processes to be managed with little variation. The use of defined cultures became the industrial standard in breweries by the 19th century. During the 20th century, the wine, dairy, and meat industries also shifted production procedures toward the use of well-characterized and defined starter cultures. The application of microbiology and process technology resulted in large improvements in the quality of the fermented food products. The quality improvements have been so great that today all significant production of fermented food is industrial, or at least professionally performed. The small amount of "home fermentations" conducted in the form of baking, home brewing, and private cheese making usually rely on commercially available yeast and bacterial cultures. The maintenance of the microorganisms differs between the different food industries in the sense that some fermentation industries such as breweries and vinegar producers maintain their own strains and inocula. In the dairy industry, as well as in the meat industry and bakeries, cultures are usually obtained from suppliers dedicated to the production of high-quality food ingredients.

III. MICROORGANISMS

A. Yeast, Molds, and Bacteria

A large variety of microorganisms have been employed in food fermentations. Yeast and mold species commonly used are listed in Table 2, and bacterial species are listed in Table 3. The two lists represent a compilation of the species found in commercially available cultures (2–10, personal communications by H. Heap and M. B. Prevot, 2002) as well as those commonly found in food fermentations (11–14). The lists are not complete with respect to species that are only occasionally found in fermented foods. Of the large number of microorganisms listed, a few are exceptionally widely used. The top three are *Saccharomyces cerevisiae*, *Lactococcus lactis,* and *Streptococcus thermophilus*.

Saccharomyces cerevisiae is used as baker's yeast, brewer's yeast, inoculums for wine fermentations, food and feed additives, and as flavor-generating cultures in dairy and meat products. The annual production of baker's yeast is approximately 1 million tons; the

Table 2 Yeast and Mold Species Commonly Used in Food Fermentations

Genus	Species	Application
Aspergillus	*oryzae*	Soy sauce
Candida	*famata*	Meat
Candida	*kefyr*	Fermented milk
Candida	*krusei*	Fermented milk
Candida	*lipolytica*	
Candida	*Parapsilosis*	
Candida	*valida*	
Geotrichum	*candidum*	Cheese, fermented milks
Penicillium	*album*	
Penicillium	*camemberti*	Cheese, meat
Penicillium	*chrysogenum*	Meat
Penicillium	*nalgiovense*	Meat
Penicillium	*roqueforti*	Cheese, meat
Saccharomyces	*bayanus*	Fermented milks
Saccharomyces	*cerevisiae*	Baker's yeast, brewing, wine-making, cheese, fermented milks, meat, vegetables, and probiotics

volume of the yeast production exceeds the combined production of all other microorganisms by about two orders of magnitude. The bulk production of yeast is a commodity quite different from the high value, low volume yeast strains produced for inoculating wine fermentations, and is also quite different from all the other inoculants for food fermentations.

Lactococcus lactis is the most widely used lactic acid bacterium. *L. lactis* is used for the production of cheese, butter, buttermilk, and other fermented milks and, to some extent, is used in meat, bread, and vegetable products.

Streptococcus thermophilus is the second most-used lactic acid bacteria. It is used in the dairy industry for the production of yogurt and a variety of other fermented milk products, and for the production of several cheeses, most notably mozzarella and pizza cheese. Due to the high acidification activity, *S. thermophilus* is often used in combination with other lactic acid bacteria to increase the speed of the fermentation.

Of the *Lactobacillus* species, *L. delbrueckii* and *L. acidophilus* are used in relatively large volumes, especially in dairy products; *L. acidophilus* is also used in various probiotic products. Several species of *Bifidobacterium* are used as probiotic cultures in fermented foods and food supplements. The other species listed in Tables 2 and 3 are produced in much smaller volumes, but for some strains the contribution to the final product is so essential that the value of the culture can be very high although the volume is small.

B. Taxonomy

Identification and classification of microorganisms has traditionally been a difficult task due to the large number of different microorganisms of relatively uniform cell morphology and colony morphology. The phenotypes used in classical microbiology are often of the +/− type. Such characters are suited for the identification of individual species but difficult to use

Table 3 Bacterial Species Commonly Used in Food Fermentations

Genus	Species	Application
Acetobacter	*aceti*	Vinegar production
Bifidobacterium	*adolescentis*	Probiotics
Bifidobacterium	*animalis*[a]	Cheese, fermented milks, probiotics
Bifidobacterium	*bifidum*	Cheese, fermented milks, probiotics
Bifidobacterium	*breve*	Probiotics
Bifidobacterium	*infantis*	Probiotics
Bifidobacterium	*longum*	Probiotics
Brevibacterium	*casei*	Cheese
Brevibacterium	*linens*	Cheese, bioprotection
Carnobacterium	*divergens*	Meat, bioprotection
Carnobacterium	*piscicola*	Meat, bioprotection
Enterococcus	*faecium*	Cheese, fermented milks, meat, vegetables, probiotics, bioprotection
Kocuria	*varians*[b]	Meat
Lactobacillus	*acidophilus*	Probiotics, cheese, fermented milks, meat, vegetables
Lactobacillus	*alimentarius*	Meat
Lactobacillus	*brevis*	Probiotics, vegetables, bioprotection
Lactobacillus	*casei*	Probiotics, cheese, fermented milks, meat, vegetables
Lactobacillus	*coryniformis*	Cheese
Lactobacillus	*crispatus*	
Lactobacillus	*curvatus*	Meat
Lactobacillus	*delbrueckii* subsp. *bulgaricus*	Fermented milks, cheese, probiotics
Lactobacillus	*delbrueckii* subsp. *delbrueckii*	Cheese, vegetables
Lactobacillus	*Delbrueckii* subsp. *lactis*	Fermented milks, cheese
Lactobacillus	*farciminis*	Meat
Lactobacillus	*fermentum*	Cheese, probiotics
Lactobacillus	*gasseri*	Fermented milks, probiotics
Lactobacillus	*helveticus*	Cheese, fermented milks, probiotics, vegetables
Lactobacillus	*johnsonii*	Fermented milks, probiotics, probiotics
Lactobacillus	*kefiri*	Fermented milks
Lactobacillus	*panis*	Sourdough, bread
Lactobacillus	*pentosus*	Meat
Lactobacillus	*plantarum*	Bread, meat, wine, vegetables, bioprotection
Lactobacillus	*reuteri*	Bioprotection, probiotics
Lactobacillus	*rhamnosus*	Probiotics
Lactobacillus	*sakei* subsp. *carnosus*	Meat
Lactobacillus	*sakei* subsp. *sakei*[c]	Meat, vegetables, bioprotection
Lactobacillus	*sanfranciscensis*	Bread
Lactococcus	*lactis* subsp. *cremoris*	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection
Lactococcus	*lactis* subsp. *lactis*	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection

Table 3 Continued

Genus	Species	Application
Lactococcus	*lactis* subsp. *lactis* biovar *diacetylactis*	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection
Leuconostoc	*carnosum*	Meat, bioprotection
Leuconostoc	*lactis*	Cheese, fermented milks
Leuconostoc	*mesenteroides* subsp. *cremoris*	Cheese, fermented milks, vegetables
Leuconostoc	*mesenteroides* subsp. *dextranicum*	Probiotics
Leuconostoc	*mesenteroides* subsp. *mesenteroides*	Cheese, fermented milks, vegetables
Leuconostoc	*pseudomesenteroides*	
Micrococcus	*luteus*	Meat
Oenococcus	*oeni*	Wine
Pediococcus	*acidilactici*	Meat, probiotics, bioprotection
Pediococcus	*damnosus*	Meat, bioprotection
Pediococcus	*pentosaceus*	Meat
Propionibacterium	*acidipropionici*	Cheese
Propionibacterium	*freudenreichii* subsp. *freudenreichii*	Cheese, probiotics
Propionibacterium	*freudenreichii* subsp. *shermanii*	Cheese
Staphylococcus	*carnosus* subsp. *carnosus*	Meat
Staphylococcus	*carnosus* subsp. *utilis*	Meat
Staphylococcus	*equorum*	Meat
Staphylococcus	*xylosus*	Meat
Streptococcus	*thermophilus*	Cheese, fermented milks, bread, meat, vegetables, probiotics
Weissella	*confusa*	Meat
Weissella	*halotolerans*	Meat

[a] *Bifidobacterium lactis* is not a separate species but included in *B. animalis*.
[b] *Micrococcus varians* has been renamed *Kocuria varians*.
[c] *Lacttobacillus bavaricus* has been included in *L. sakei* subsp. *sakei*.

in organizing species into higher orders of relatedness. The traits traditionally used for identification are cell morphology, Gram staining, growth on various carbohydrates, gas formation, acid production, temperature profile for growth, salt tolerance, amino acid requirements, vitamin requirements, oxygen requirement/sensitivity, hemolysis, and hydrolysis of polysaccharides, proteins, and lipids. At the strain level, methods for discrimination are based on serological tests, phage typing, or very specific biochemical tests. All these traditional tests are still used and important in the microbiology laboratory, particularly those relating directly to the metabolisms exploited for the fermentation process. Identification, classification, and taxonomy of microorganisms have, however, undergone dramatic changes during the past two decades due to the introduction of methods from molecular biology (15). These methods have allowed us to base identification and taxonomy on the properties common to all microorganisms instad of the traits that differ. Several of the fundamental processes in a living cell are shared by all organisms, and one of

these is protein synthesis. The ubiquitous presence of ribosomes allows taxonomists to use their degree of similarity to deduce evolutionary distances between organisms. For practical reasons the RNA molecules of the ribosome are particularly convenient for this purpose (16, 17). The phylogenetic relationships between the species listed in Table 3 are also presented in Fig. 1. A few G^+ pathogens, as well as the G^- *Escherichia coli*, have been included for reference, whereas a large number of other bacterial species have been omitted for purposes of clarity. It has previously been pointed out that species of lactic acid bacteria do not have a close phylogenetic relationship, but share a common strategy for survival in nutrient-rich environments (15). By inspecting Fig. 1, it is apparent that further changes in the taxonomy of the *Lactobacillus-Pediococcus* group will be necessary before the taxons can be represented in clades. Fig. 1 also clearly illustrates how small the evolutionary distance can be between beneficial and lethal microorganisms.

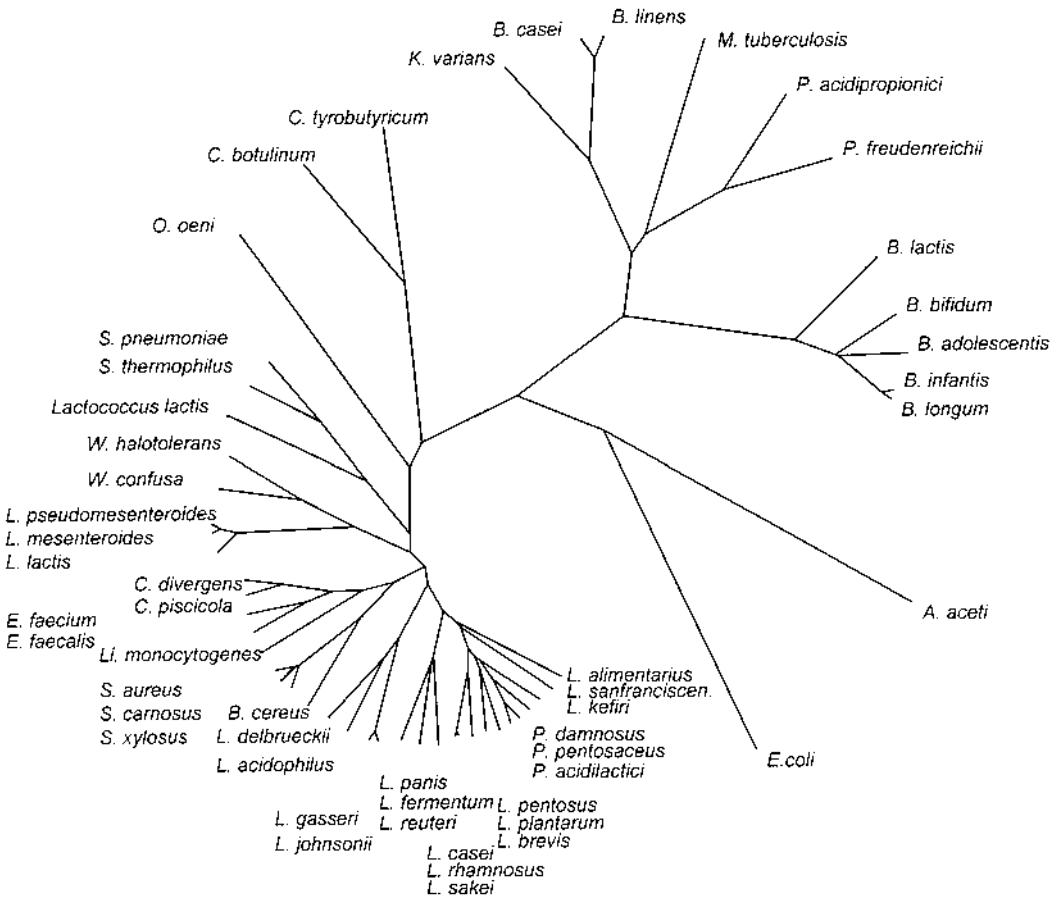

Figure 1 The phylogenetic relationship between the bacterial species listed in Table 3. The phylogenetic tree was constructed based on the 16S rRNA sequences using the server of the ribosomal database project (*http://rdp.cme.msu.edu*, 17). The G^- species, *Escherichia coli*, has been included for reference. Also included as references are a few G^+ pathogens and spoilage organisms: *Mycobacterium tuberculosis*, *Clostridium tyrobutyricum*, *Clostridium botulinum*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*.

General methods for identification below the species level are now also based on molecular methods. DNA fingerprint methods able to identify individual strains have been developed; one of the most reliable and portable methods uses pulsed field gel electrophoresis of restriction patterns of entire bacterial chromosomes (18).

IV. PERFORMANCE PARAMETERS

A. Metabolism, Activity, and Reliability

The currently employed food fermentations rely on only a few main metabolic pathways:

Anaerobic alcoholic fermentation, which converts carbohydrates to alcohol and CO_2
Lactic fermentation, which converts carbohydrates to lactic acid
Aerobic acetic acid fermentation, which converts alcohol to acetic acid

The main end products—alcohol and organic acids—are responsible for the primary preservation of the foods, and they also contribute significantly to the flavor of the fermented products. Secondary metabolic pathways do, however, create a large variety of flavors and textures, yielding a diversity of products (11). To satisfy their need for nutrients, microorganisms are able to produce hydrolytic enzymes such as proteases, peptidases, lipases, glucanases, amylases, and so forth. In addition to satisfying the microorganism's needs, these enzymes also have the potential to generate or degrade strongly flavored products (19–23). Enzymatic reactions are very important in ripened meat products and cheese. The substrate specificity of enzymes varies considerably among microbial species and strains, and this is an important source of the diversity of starter cultures. The accumulation profile of small flavored metabolites such as diacetyl, acetaldehyde, acetate, and formate also contributes significantly to the diversity of cultures. The balance between these metabolites is determined by the precise metabolic route leading from the carbohydrate to main end products, as well as the utilization of alternative electron acceptors (15). Not only generation of flavor, but also generation of gas and texture, is dependent on the subtle differences between the metabolic routes of the cultures. These differences are responsible for the presence or absence of eyes in cheeses, bubbles or not in fermented milk, and so forth.

A suitable metabolism is, as just described, the primary performance parameter for a starter culture, but next in importance is speed and reliability. The optimization of the fermentation speed involves reducing the lag phase, increasing the growth rate, and increasing the metabolic activity of the cells. Reliability is obtained by selecting strains with low sensitivity to environmental factors that can be encountered in a particular fermentation. A general problem in large-scale fermentations is bacteriophage attack of the starter culture. This has been a particular problem in the cheese industry due to the repeated use of open vats, which cause severe losses. Considerable research efforts have been directed toward the protection of *Lactococcus lactis* from attacking phages. These efforts have been successful in generating new fundamental knowledge about bacteriophages and in generating new bacteriophage resistance mechanisms (24,25).

B. Biopreservation

A general preservation effect is obtained by most food fermentations from the accumulation of organic acids and alcohols concomitantly with the reduction in free sugar levels, depletion of oxygen, and lowering of the pH (26). Cultures with much stronger preservation effects

have been identified and in most cases have been found to produce antimicrobial bacteriocins (12). *Lactobacillus reuteri* is an interesting exception because its antimicrobial substance, reuterin, is a low molecular weight metabolite, 3-hydroxypropionaldehyde (27). The first bacteriocin, nisin, was discovered about 70 years ago. Nisin is produced by strains of *Lactococcus lactis*, and the molecule is a small peptide containing unusual amino acids due to posttranslational modifications (28). Nisin has been in practical use as a food preservative for more than 50 years, and its use is approved in most countries. A large number of bacteriocins have been characterized from lactic acid bacteria and are classified into three groups based on their structural differences (12). The bacteriocins share a common mode of action in their ability to form pores in the membrane of the target bacteria; the molecular aspects of the formation of pores have been well characterized, particularly for nisin (29,30). The ability to produce bacteriocins is quite common among microorganisms isolated from fermented foods, and the consensus among all studies is that this property is beneficial and safe (12,14,31). It is, therefore, to be expected that a number of bioprotective cultures will be introduced into the market.

C. Probiotic Effects

The beneficial effect of lactic acid bacteria on human health was described by Metchnikoff almost a century ago (32). Several studies have substantiated these positive health effects, which was later named the probiotic effect. The currently used definition of probiotics is as follows: "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (33). It has, however, turned out to be difficult to identify and prove the mode of action for probiotics (34,35). The bacterial flora of the digestive tract is an extremely complex ecosystem consisting of numerous bacterial species (36–38). The intestinal microbial flora is necessary for the normal function of the digestive system. Elimination or severe perturbations of the flora leads to diarrhea or constipation; therefore, the maintenance of healthy bacterial flora is desirable (35,36). In absence of a well-defined mode of action for probiotics, practical criteria for selecting probiotic strains have been formulated (34,39–41). The main requirements are acid and bile stability, antagonism toward pathogenic bacteria, safety in use, and clinical documentation of the health effects.

V. COMMERCIALLY AVAILABLE STARTER CULTURES

A. Production of Starter Cultures

The propagation of microorganisms on an industrial scale is a central and most obvious part of the production of starter cultures. Depending on the product, industrial scale can range from laboratory propagation in flasks and agar plates to fermentors of hundreds of cubic meters in size. Except for a few of the mold cultures produced by sporulation on solid media, most cultures are produced by liquid submerged fermentation. Aerobic fermentations are used for the production of yeast and aerobic flavor cultures; however, the majority of the bacterial cultures are produced by anaerobic fermentations. Less obvious and therefore probably more important for the quality of the culture products are the procedures occurring upstream or downstream relative to the fermentation. Among the upstream processes, the most important ones are those that secure the identity and purity of the microorganisms produced. In order to eliminate the risk of a gradual change of the product over time due to genetic instability of the microorganism, the internal production of inocula must be organized so that each batch has the same "molecular" age (42). The

identity of the inoculum must also be verified, preferably by DNA fingerprinting methods (43).

The procedures downstream from the fermentation are designed to increase the cell density, to preserve the microorganisms, and to package the products in a format allowing convenient storage, distribution, and use. The cell density can be increased by centrifugation or ultra filtration. Depending on the desired format of the product and the fragility of the microorganism, the product can be packaged in liquid, dry, or frozen form. The packaging material must be designed to protect the microorganisms from excessive heat, moisture, and light. The actual sensitivity to these factors can vary considerable among different culture types, but also between different formulations of the same culture.

Typical production processes for starter cultures have been described by Høier et al. (44). Culture producers use similar principles for quality assurance and HACCP (hazard analysis critical control point) in the production processes (45).

B. Formats and Formulations of Cultures

Food fermentations can be inoculated either directly with a highly concentrated starter culture obtained from the supplier, or they can be inoculated from a bulk starter propagated locally. The choice between the two process types will be influenced by a number of factors: the number of different fermented products produced in the same factory, degree of automation, presence of expertise in microbiology and, finally, the economics. The highest level of safety and flexibility is achieved by direct inoculation of the culture. In addition to the choice between direct inoculation or bulk starter, there are choices between different culture formulations. The usual options are fresh, dried, or frozen, but their availability differs between suppliers and products.

Baker's yeast is generally inoculated directly into the dough without propagation in the bakery. Yeast is supplied in both fresh and dry forms; fresh yeast can be obtained as liquid, compressed, or crumbled yeast, and dry yeast either as active or instant yeast (8,9,46). Mold cultures are mainly used as direct inoculants, and the common format is a dry spore preparation (2,5,7).

Bacterial cultures are sold as liquid, lyophilized, or frozen cultures. Liquid bacterial cultures will generally lose activity within days, and for direct cultures this format will require a constant supply. Lyophilized or frozen cultures maintain high activity for months or even years, and these formats are ideally suited for global distribution of direct inoculants. Bulk systems for factory propagation of cultures are common for large-scale cheese productions. The cultures used in these systems are supplied from a starter manufacturer in frozen or freeze-dried form, and the media to be used for the propagation is available from the same source (3–10).

C. Quality and Safety of Industrially Produced Cultures

Safety is probably the main reason to buy starter cultures from a commercial supplier rather than propagating local stocks of cultures. The commercial suppliers have the scientific and technical competencies necessary to allow them to maintain purity with respect to cross-contamination as well as protection against harmful contaminants. The preservation of the cultures in lyophilized or frozen form allows the time required to perform extensive quality control, including analyses for activity, identity, and purity. Also, the regulatory competencies are important to assure compliance with the relevant national and international legislation and standards, not only for the culture manufacturer but also for the consumers

Table 4 Range of Starter Cultures from Commercial Suppliers

		Bakery	Wine		Meat		Cheese			Ferm.	Other	
		Y	Y	B	B	M&Y	B	R	M&Y	B	Pro.	BioP.
Alce	Italy						+	+	+	+	+	
ASCRC	Australia						+			+	+	
Chr. Hansen	Denmark			+	+	+	+	+	+	+	+	+
CSK	Netherlands						+	+		+	+	
CSL	Italy						+	+	+	+	+	
Danisco	Denmark						+	+	+	+	+	+
Degussa	Germany				+	+	+	+	+		+	
DSM	Netherlands	+					+			+	+	
Gewürzmüller	Germany				+	+						
Lallemand	Canada	+	+	+			+			+	+	
NZDRI	New Zealand						+	+	+	+	+	
Quest International	United Kingdom				+		+			+	+	+
Rhodia	France						+	+	+	+	+	+

(For each food industry the availability of the different culture types is indicated by a +.) The culture types are yeast (Y), bacteria (B), mold and yeast (M&Y), bacterial ripening cultures (R), probiotics (Pro.), and bioprotective cultures (BioP.). Ferm. designates fermented milk.

of the food. The safety issue will often lead food manufacturers to have even their own proprietary cultures produced by one of the suppliers.

D. Suppliers of Starter Cultures

The following survey contains suppliers engaged in production and development of starter cultures. Manufactures of standard yeast products have not been included. Companies producing starter cultures range in size from small companies supplying cultures only to subscribing food industries to large global companies supplying these and other food ingredients.

An overview of the culture ranges is given in Table 4 for small as well as large companies; the range of other food ingredients is outside the scope of this chapter. The country of origin is also included in Table 4, and all companies can easily be found on the worldwide web.

VI. INNOVATION TRENDS

Innovation in the starter culture industry is stimulated by possibility and need. New possibilities are constantly being opened up by rapid developments in the biological sciences. Our ability to understand complex biological systems has been transformed through the invention of methods to accumulate and analyze large amounts of data. The genomes of more than 100 microorganisms have now been completely sequenced, including several pathogenic bacteria as well as some of the microorganisms used in food fermentations [e.g., *Saccharomyces cerevisiae* (47) and *Lactococcus lactis* (48)]. Safe methods for the genetic engineering of food microorganisms have been developed for the most important species,

and this has opened a wide range of possibilities for the improvement of yeast and lactic acid bacteria metabolism (49–54). The practical applications of the modern methods in Europe, however, have been delayed due to public resistance to modern biotechnology.

The other factor stimulating innovation is the need for new products. There is a big need for new methods to preserve crops after harvest, to reduce spoilage before consumption. This need is very strong in the less developed world, but also in the highly developed countries do we need better methods to extend shelf life and avoid spoilage. Food fermentation and bioprotective cultures can solve some of these problems. Probiotic cultures with specific health benefits, with defined modes of action, is also an area where the market would welcome new products. These two examples are specific areas under the more general need for new cultures or new culture formulations in order to expand the use of beneficial microorganisms in food.

ACKNOWLEDGMENTS

I am grateful to Dr. Howard Heap (New Zealand Dairy Research Institute), Dr. Claudio Rottigni (Centro Sperimentale del Latte), Dr. Michela Bianco Prevot (Alce), Dr. Francette Hamaide (Degussa), Dr. Odile Conan (CSK food enrichment), Dr. Gaëtan Limsowtin (ASCRC), Knud Vindfeldt (Chr. Hansen A/S), and Danny O'Regan (Danisco A/S), for providing information on product ranges and bacterial species in commercial use.

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3
Starter Cultures and Fermented Products

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I. INTRODUCTION

Fermentation is a process in which microorganisms, in the absence of oxygen, generate energy by oxidizing carbohydrates and related compounds. It has been used since ancient times as an important method for preserving food. Vegetables, fruits, cereals, milk, and other raw materials have been treated in special ways in order to promote the growth of beneficial microorganisms while inhibiting the growth of deteriorating and pathogenic microorganisms. Fermentation will preserve the food, and it will also enhance the taste, aroma, texture, and nutritional value of the product. The preservation effect is the result of synthesis of lactic acid and heterofermentation—also, acetic acids and, some times, antimicrobial substances. Besides lowering the pH level, organic acids are also toxic for many microorganisms. It is also important that the fermentable carbohydrates are completely utilized by the fermenting microorganisms and thereby made unavailable for the undesirable microorganisms. In some products the addition of salt will increase the shelf life of the products by lowering the water activity. The natural habitats of lactic acid bacteria, yeast, and molds are most often plant materials. However, the type of organisms can vary considerably (1), depending on type of plant, climatic conditions, and available nutrients in the raw material. During some fermentations (e.g., fermentation of plant material such as cabbage, cucumbers, olives, soya beans, and coffee), several different types of microorganisms are required at the various stages of the fermentation process. In other fermentations (e.g., production of yogurt and beer), only a few different microorganisms are required.

Even though the involvement of microorganisms and their importance for the fermentation process was not known until relatively recently, it was found by practice that for some fermentations the addition of a portion of a previous fermentation was beneficial for the process. With the utilization of pasteurization of milk in the late 19th century, it was discovered that bacteria were necessary for the souring of milk for production of butter. In 1878, Joseph Lister isolated a pure culture from sour milk and named it *Bacterium lactis* (2). In 1919 Orla-Jensen classified this bacterium as *Streptococcus lactis* (see Fig. 1) (3); today it is classified as *Lactococcus lactis* subsp. *lactis* (4). Shortly after this discovery, Vilhelm Storch in Denmark, Herman Weigmann in Germany, and H.W. Conn in the United States (5) independently introduced the addition of pure cultures to milk in order to improve fermentation. In 1896 Storch was granted a U.S. patent on production of

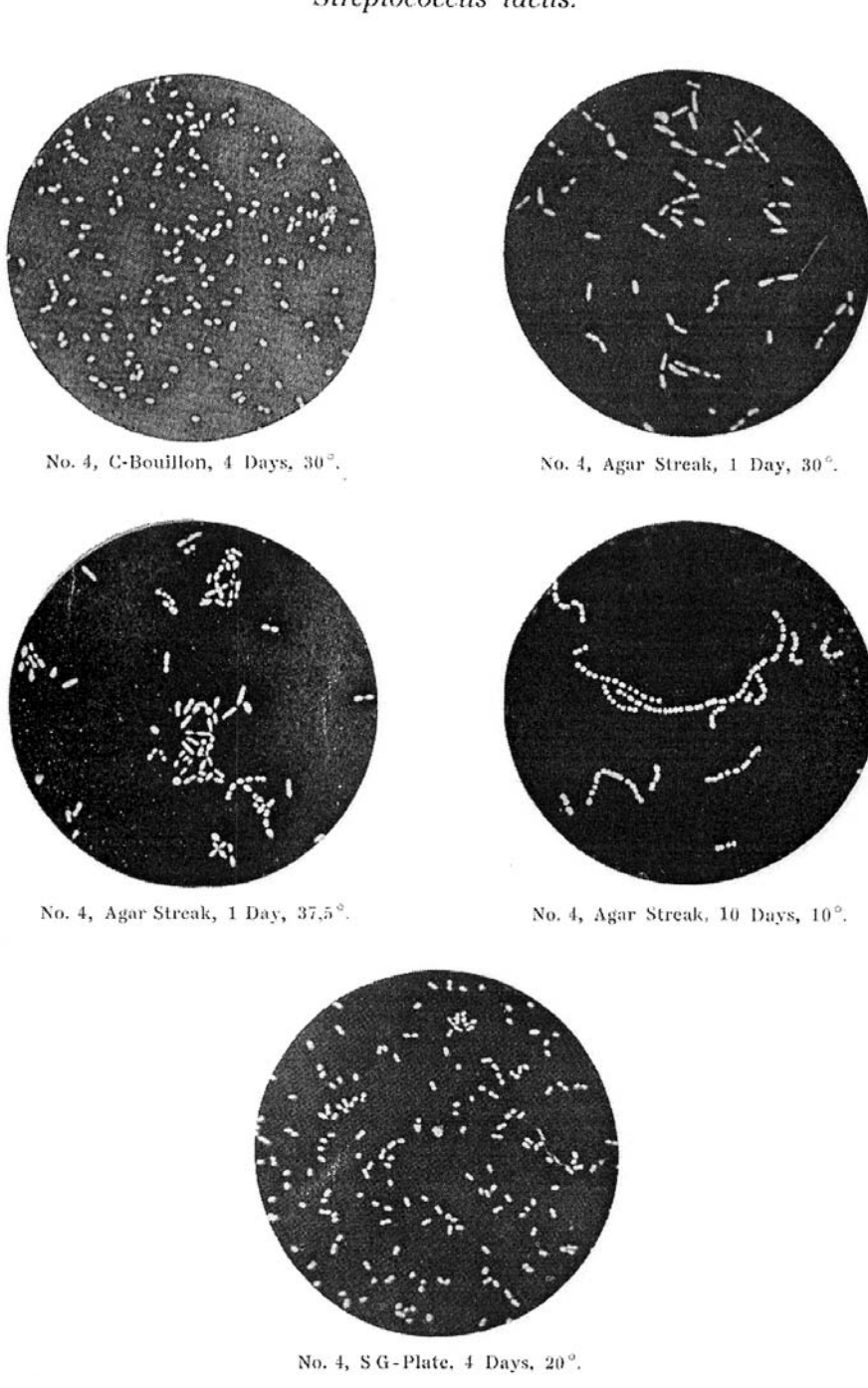

Figure 1 Micrograph of *Lactococcus lactis* subsp. *lastis* (original *Streptococcus lactis*) made by S. Orla-Jensen in 1919. (From Ref. 3:) Magnification: 1000×.

starter cultures. Most of the bacteria used for milk fermentations were named lactic acid bacteria (LAB) because they mainly produce lactic acid by their catabolism of the milk sugar (lactose); and because they were used to start or begin the fermentation, they were termed starters or starter cultures (6).

A breakthrough in the history of wine occurred when Louis Pasteur described "life without oxygen" and of brewing fermentation when Emil Christian Hansen at Carlsberg brewery in the late 19th century isolated a pure yeast culture from single cells. For wine making, the concept of inoculating wine fermentations with pure yeast starter cultures was introduced by Müller-Thurgau in 1890, and in 1965 the first commercial dried starter cultures were produced for a large Californian winery (7). In 1920 the "fed-batch" process was introduced for the production of baker's yeast, and that resulted in a significant increase in the industrialized production of bakeŕs yeast (8). The involvement of molds (mycelial fungi) in food fermentation goes back to the first records on blue and white molded cheeses. The first records for the production of well-known cheeses such as Gorgonzola and Roquefort date to 879 and 1070 respectively (8). Eventually, the cultures involved in the fermentation were purified and starter cultures are now commercially available for cheese and meat even though not developed to a great extent.

Over the years, the concept of utilization of starter cultures for the production of fermented food, especially of bread, beer, fermented milk products, and cheeses, and for the production of sausages has gained increased attention.

II. FERMENTATION PROCESSES

Fermentations can basically be performed either by spontaneous fermentation, by back-slopping, or by addition of starter cultures. By spontaneous fermentation the raw material, and its initial treatment, will encourage the growth of an indigenous flora (9). For most spontaneous fermentations, a microbial succession takes place: quite often LAB will initially dominate, followed by various species of yeasts. Molds will only grow aerobically, which limits their occurrence in certain types of fermented products. LAB produce lactic acid and other antimicrobial substances that will inhibit the growth of harmful bacteria, along with reducing the sugar content, thereby prolonging the shelf life of the product. Yeasts mostly produce aroma components and alcohols (10). When molds are involved in fermentation, they generally contribute by producing both intra- and extracellular proteolytic and lipolytic enzymes that highly influence the flavor and texture of the product (8).

In back-slopping, a part of a previous batch of a fermented product is used to inoculate the new batch. This procedure produces a higher initial number of beneficial microorganisms than found in raw material and ensures a faster and more reliable fermentation than occurs in spontaneous fermentation. This procedure probably also favors the growth of bacteria producing antimicrobial substances, ensuring the growth of the same bacteria every time. Examples of back-slopping are home-made fermentation of milk, vegetables, and cereals. Bread production made with sourdough is often also done by back-slopping; a sample of the previous days' sourdough is used to inoculate a new batch of dough (8).

Addition of starter cultures is most often used when it is possible to inactivate the indigenous flora by heat treatment of the raw material, permitting the growth of only the added starter microorganisms. However, it is not always possible to heat-treat the raw material (e.g., fruits and vegetables) without influencing the texture of the final product (11). Nevertheless, the addition of starter cultures—especially those containing a bacteriocin-producing strain alone or in combination with selected bacteriocin-resistant strains—may in

Table 1 Definitions of Starter Cultures

Name	Contents
Single-strain starter	A single well-defined strain with known technological properties
Multiple strain starter	2–6 well-defined strains with known technological properties
Mixed-strain starter	An unknown number of undefined strains

fermentations of plants yield a greater possibility that the desirable flora will dominate in the fermentation (12–14). Starter cultures can basically be classified as shown in Table 1.

Single-strain starter cultures are primarily used for yeasts and molds in the production of beer and wine, and LAB for the production of a few dairy products, sausages, and sauerkraut. Multiple starter cultures are used for dairy products, sourdough, sausages, and wine. Mixed undefined bacterial starter cultures, also called traditional or artisanal starters (15), are primarily used in the dairy industry and in sourdough production.

III. BACTERIAL STARTER CULTURES

A. Introduction

Starter cultures can also be classified according to their optimal growth temperature as shown in Table 2. The most important bacteria for food fermentation are designated lactic acid bacteria (LAB). They are gram-positive rods or cocci, non–spore formers, catalase negative, obligatory fermentative, microaerophilic, usually nonmotile bacteria having extensive growth requirements. They produce mainly lactic acid from glucose fermentation. The first classification of LAB was made in 1919 by S. Orla-Jensen (3). However, utilization of DNA technology and molecular typing methods has had a great impact on the taxonomy of bacteria and has led to a great deal of taxonomic revision. Table 3 shows a list of common LAB that have received new names within the past two decades. Table 4 shows the genera into which the LAB are currently divided. Table 4 also shows which pathways LAB use for fermentation of glucose. Homofermentative LAB mainly make lactic acid as their final product, whereas heterofermentative LAB make equal amounts of lactic acid, acetic acid, and CO_2. Other bacteria such as *Acetobacter, Bifidobacterium, Micrococcus,* and *Staphylococcus* are also used as starter cultures for food and beverage fermentations; *Brevibacterium* and *Propionibacterium* are used as secondary or adjunct cultures. Some *Lactobacillus* species are also used as adjunct cultures.

B. Cultures for Milk Fermentation

The utilization of starter cultures in industrial milk fermentation is widespread. The most important starter cultures are the LAB. These cultures often consist only of mesophilic or

Table 2 Types of Starter Cultures

Type	Optimum temperature	Typical growth temperature
Mesophilic	25–34°C	18–30°C
Thermophilic	37–44°C	40–44°C

Table 3 Name Changes of Common Lactic Acid Bacteria Resulting from Taxonomic Revisions[a]

New names	Old names
Carnobacterium divergens	*Lactobacillus divergens*
Carnobacterium piscicola	*Lactobacillus carnis*
Lactobacillus delbrueckii subsp. *bulgaricus*	*Lactobacillus bulgaricus*
Lactobacillus delbrueckii subsp. *lactis*	*Lactobacillus lactis*
Lactobacillus rhamnosus	*Lactobacillus casei* subsp. *rhamnosus*
Lactobacillus sakei	*Lactobacillus sake* and most strains of *Lactobacillus bavaricus*
Lactobacillus sanfranciscensis	*Lactobacillus sanfrancisco*
Lactococcus lactis subsp. *cremoris*	*Streptococcus cremoris*
Lactococcus lactis subsp. *lactis*	*Streptococcus lactis*
Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	*Streptococcus diacetylactis*
Leuconostoc mesenteroides subsp. *cremoris*	*Leuconostoc citrovorum*
Leuconostoc mesenteroides subsp. *dextranicum*	*Leuconostoc dextranicum*
Oenococcus oeni	*Leuconostoc oeni*
Streptococcus thermophilus	*Streptococcus salivarius* subsp. *thermophilus*
Tetragenococcus halophilus	*Pediococcus halophilus*
Weissella confusa	*Lactobacillus confusus*
Weissella paramesenteroides	*Leuconostoc paramesenteroides*

[a] Not all listed bacteria are used as starter or adjunct cultures; some have only been isolated from fermented food and beverage products. An updated list is present on http://www.bacterio.cict.fr.

Table 4 Genera of Lactic Acid Bacteria

Name	Old name	Number of species	Sugar fermentation
Lactococcus	Lactic or group N *Streptococcus*	5	Homo
Enterococcus[a]	Fecal *Streptococcus*	14	Homo
Streptococcus[b]		39	Homo
Leuconostoc	*Betacoccus*	9	Hetero
Oenococcus	*Leuconostoc*	1	Hetero
Pediococcus		6	Homo
Tetragenococcus	*Pediococcus*	1	Homo
Lactobacillus		>60	Group I: Homo Group II: Facultative hetero[c] Group III: Hetero
Carnobacterium	*Lactobacillus*	6	Homo
Weisella	1 previously *Leuconostoc*, 5 previously *Lactobacillus*	7	Hetero

Homo = homofermentative; hetero = heterofermentative.

[a] Several are pathogenic.

[b] Many are pathogenic.

[c] Ferment glucose by the homofermentative pathway and pentoses and 6-P-gluconate by the heterofermentative pathway.

thermophilic LAB; however, mixtures can also occur. Mesophilic starter cultures originate from north and east Europe. They consist primarily of *Lactococcus lactis* subsp. *cremoris* (*L. cremoris*), *Lactococcus lactis* subsp. *lactis* (*L. lactis*), *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (*L. diacetylactis*), *Leuconostoc mesenteroides* subsp. *cremoris*, and *Leuconostoc lacti*s. Especially, *L. cremoris* and *L. cremoris* are capable of rapid acidification of milk. *L. diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, and *Leuconostoc lactis* can catabolize citrate into CO_2 and diacetyl. CO_2 is responsible for the production of holes in the cheeses, and diacetyl, the characteristic flavor of butter, is important for the flavor of many cheeses and fermented milk products. The diacetyl-producing organisms are often called the aroma producers or *L. lactis* subsp. *lactis* (citrate +). *Lactobacillus paracasei* and *Lactobacillus casei* are the most common mesophilic lactobacilli found in many cheeses and are in some cases used as adjunct cultures (16–19). *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus curvatus* are also found in many cheeses (17,18,20). The dairy propionibacteria *Propionibacterium shermanii* and *Propionibacterium freudenreichii* are used in some Swiss-type cheeses such as Emmental, Gruyère, and Comté, in which they slowly catabolize lactate to propionate, acetate, and CO_2. This is important for the production of holes and taste of the cheeses (20,21). Micrococcacaceae and *Brevibacterium* are used as surface flora in various cheeses (22); they are important for cheese ripening.

Different types of mixed starter cultures have been developed. The composition of the different mesophilic starter cultures, and examples of products for which they are used, is shown in Table 5. The most abundant cheese produced is Cheddar cheese. It is commonly produced by the use of a multiple strain starter culture of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*, with or without *Streptococcus thermophilus*. However, a mixed O-culture, TK5, has also been developed (23,24).

Table 5 Composition of Different Types of Mesophilic Starter Cultures and Some Examples of Their Products

Type	Organisms	Composition	Products
O	*Lactococcus lactis* subsp. *lactis*	5–10%	Cheddar
	Lactococcus lactis subsp. *cremoris*	90–95%	Cottage cheese Feta Quarg
L	*Lactococcus lactis* subsp. *lactis*	5–10%	Lactic butter
	Lactococcus lactis subsp. *cremoris*	80–90%	Feta
	Leuconostoc mesenteroides subsp. *cremoris* *Leuconostoc lactis*	5–10%	Cheddar
D	*Lactococcus lactis* subsp. *lactis*	5–10%	Lactic butter
	Lactococcus lactis subsp. *cremoris*	70–85%	
	Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	10–20%	
DL	*Lactococcus lactis* subsp. *lactis*	5–10%	Continental cheese (with eyes)
	Lactococcus lactis subsp. *cremoris*	60–80%	Mold-ripened cheese
	Lactococcus lactis subsp. *lactis* biovar. *diacetylactis*	10–20%	Lactic butter Cultured buttermilk
	Leuconostoc mesenteroides subsp. *cremoris* *Leuconostoc lactis*	5–10%	Creme fraiche, ymer

Thermophilic cultures originate from south and east Europe. They consist mainly of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *lactis,* and *Lactobacillus helveticus*. The thermophilic LAB are used for rapid acidification or as adjuncts in cheeses. *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus,* and some *Lactobacillus delbrueckii* subsp. *lactis* catabolize lactose into lactate and galactose, which are secreted. The residual galactose can create problems (e.g., growth of undesirable bacteria) in cheese and influences the browning of pizza cheese. *Lactobacillus helveticus* can use galactose as a carbon source and thereby remove the residual galactose. Furthermore, some strains of *Lactobacillus helveticus* are very proteolytic, thereby influencing the taste and texture of cheese (25,26). Thermophilic LAB can also produce acetaldehyde, which is the characteristic flavor of yogurt. The composition of these starter cultures varies. Both defined single or multiple starter strain cultures and mixed undefined cultures are used. Mozzarella and yogurt are commonly produced by single or multiple strain starters that contain one or more *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains, but many cooked cheeses are produced with mixed cultures. They are used either as milk cultures or whey cultures, with or without rennet. Thermophilic cultures and some examples of their products are described in more detail in Chapter 16.

Other cheeses or fermented milk products are made with both mesophilic or thermophilic LAB starter cultures, or by addition of other LAB such as *Enterococcus faecium, Lactobacillus acidophilus*, or other genera of bacteria (e.g., *Bifidobacterium*). Yeast and mold can also be added. Table 6 gives examples of dairy products produced with different combinations of microorganisms.

One of the disadvantages of using pure bacterial starter cultures is that they are more sensitive to bacteriophages than undefined mixed starters (23) or indigenous flora, where there will always be strains present that can survive phage attack and continue fermentation. Lactic acid fermentations of milk for cheese production are especially susceptible to phages, and special precautions have to be taken in order to exclude them. The use of phage-resistant starter cultures, a high level of cleaning and sanitation, use of closed vats equipped with filters, specially designed pipelines, equipment and facilities, minimal access of persons, and high personal hygiene are all recommended to achieve this. In this way, it was possible to use a mixed O-culture, TK5, for production of Cheddar cheese in Denmark for 11 years before bacteriophages able to inhibit acidification appeared (27,28). Unfortunately, the phages became so virulent that after 12 years of production, it was not possible to use the TK5 starter culture any longer in the dairy (23).

Table 6 Examples of Fermented Milk Products and the Composition of their Added Starter Cultures

Fermented milk products	Microorganisms
Acidophilus milk	DL starter, *Lb. acidophilus*
Viili	DL starter, *Geotricium candidum*
Langfil	D, L, or DL culture
Cultura, AB-milk	*Lb. acidophilus*, *Lb. johnsonii*, *Lb. gasseri*, *Bifidobacterium*
Yogurt variants	*S. thermophilus*, *Lb. acidophilus*
Yogurt variants	*S. thermophilus*, *Bifidobacterium*
Gaio	*S. thermophilus*, *E. facium*
Yakult	*Lb. casei*

C. Cultures for Fermentation of Vegetables, Fruits, and Grains

Plant fermentations involve either lactic acid, acetic acid, or alcoholic fermentation or a combination of these fermentation types. In alcoholic fermentation, it is mainly yeast (*Saccharomyces cerevisiae*) and fungi (*Aspergillus oryzae*) that participate; however, lactobacilli and *Pediococcus* can also be involved. This fermentation is described in more detail in Sec. IV. Acetic acid fermentation, used for production of vinegar, is a two-stage fermentation process in which the first stage includes an alcoholic fermentation followed by the oxidization of ethanol via acetaldehyde to acetic acid (29). The typical raw materials are grapes, potatoes, or rice. Different subspecies of Acetobacter (*A. acati*, *A. pasteurianus*, and *A. hansenii*) and *Gluconobacter oxydans* are used for vinegar production. Pure cultures are not widely employed in the acetic acid fermentation industry (29,30). Interestingly, Nanda et al. (31) found that the *Acetobacter* strain responsible for the rice vinegar "Komesu" and "Kurosu" spontaneously established an almost pure culture during its long production time.

Traditional fermentations of vegetables, fruits, and grains most often include a lactic acid fermentation involving many different species of LAB that are active at different stages of the fermentation process; this is followed by fermentation by yeast and mold (30,32–34). *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are the major microorganisms; however, many other LAB (e.g., *Lactobacillus* species and *Pediococcus*) may be involved.

Fermentation of vegetables is difficult to control (35) because it depends on the quality of the raw material, the harvesting condition, and the temperature, which are vital in providing the optimal conditions for growth of the desirable microorganisms. The addition of salt, either as dry salt (2–3% w/v) or in solution (4–10% w/v) (called brining) and the creation of an anaerobic condition is commonly the initial step in fermentation of vegetables. One obstacle is that raw vegetables cannot normally be pasteurized without adverse effects on the product texture (11). Another issue is that vegetable fermentation often relies on a very complex process in which many different bacteria succeed each other in very specific ways.

Many different plant fermentations are commercially produced (35) and most often on a small scale. Currently only olives, pickled cucumbers, sauerkraut, and kimchi are industrially produced in economically important large amounts (36). Commonly, the fermentations are performed by spontaneous fermentation or back-slopping. In a few cases, utilization of LAB as single-strain cultures has been tried successfully. For production of sauerkraut, *Lactobacillus plantarum* (37,38), *Lactobacillus curvatus* (37), and *Leuconostoc mesenteries* alone (30) or combined with *Lactococcus lactis* (39) have been tried. *Lactobacillus plantarum* and *Lactobacillus pentosus* have been used in olive fermentation (40–44); *Lactobacillus plantarum* (45–48), *Lactobacillus pentosus* (48), and *Pediococcus pentosaceus* (48) successfully for pickled cucumbers. Examples of plant fermentations in which starter cultures have been used are shown Table 7.

Different grains [e.g., maize, rice, sorghum (49–64)] and legumes [e.g., soybeans, lupins, peas, lentils (65)] can be fermented, and most do not involve addition of starter cultures but are fermented by spontaneous fermentation or back-slopping. However, starter cultures are used for production of sourdough from wheat or rye (30). They are used as either single- or multiple-strains starters, with or without the addition of yeast. The LAB used in starter cultures are shown in Table 7. However, back-slopping using a batch of dough derived from a previous fermentation to inoculate the next batch of dough is still a common practice in industrial production in Denmark, Finland, and Germany. Another

Table 7 Examples of Lactic Acid Bacteria Used as Starter Cultures or Occurring Spontaneously in High Number in Fermented Plant Material

Raw material	Dominating microorganisms or starter culture	Products
Cabbage	*Leuconostoc mesenteroides*,[a] *Lb. plantarum*,[a] *Lb. curvatus*,[a] *Lb. brevis, P.cerevisiae*	Sauerkraut
Cucumber	*Lb. brevis*, *P. cerevisiae*, *Lb. plantarum*,[a] *Lb. pentosus*,[a] *P. pentosaceus*, yeast	Salted/pickled cucumber
Olives	*Lb. brevis*, *P. pentosaceus*, *Lb. plantarum*,[a] *Lb. pentosus*, yeast	Olives
Fruit juice	*Lb. casei*, *Lb. plantarum*, *Lb. xylosus*, *Lb. sakei*	Fruit juice
Wheat and rye	*Lb. sanfranciscensis*,[a] *Lb. brevis*,[a] *Lb. plantarum*,[a] *Lb. fermentum*,[a] *Lb, fructivorans*,[a] *Lb. delbrueckii*[a]	Sourdough

[a] Have been used as starter culture.

way to start the fermentation is by addition of dried dough (66). Many different LAB (especially *Lactobacillus* species) have been isolated from sour dough (67–69).

D. Cultures for Meat Fermentation

Starter cultures for meat fermentation are mainly used in the production of fermented sausages. These cultures are either single- or multiple-strain cultures of LAB and/or staphylococci. However, sausages may also be produced without the addition of starter

Table 8 Examples of Fermented Meat Products and Composition of Their Added Starter Cultures

Products	Microorganisms	Comments
Semidry sausages	*Staphylococcus carnosus*	
	+/− *Lactobacillus pentosus*	
	+/− *Pediococcus pentosaceus*	
Dry sausages	*Staphylococcus xylosus*	
	± *Pediococcus pentosaceus*	
Special cultures	*Lactobacillus pentosus*	
	Lactobacillus sakei	Bioprotection
	Pediococcus pentosaceus	
	Pediococcus acidilactici	High temperature
	Pediococcus acidilactici	Enhanced safety
	Lactobacillus curvatus	
	Lactobacillus plantarum	[a]
	Lactobacillus sakei	[a]
	Lactobacillus curvatus	[a]

+/−, with or without.
[a] Source: Refs. 30 and 73.

cultures, relying instead on the indigenous microflora in the meat (70). However, starter cultures provide technological advantages such as rapid and uniform acidification, good texture and slice-ability, production of desirable flavor compounds, enhanced safety (71,72), good color formation and stability, and better control over the fermentation process. Staphylococci are important for the aroma and color formation and stability; LAB are central for the other properties. Because meat contains extremely low amounts of sugar, the addition of carbohydrate influences the final pH. There are two main types of sausages, the northern European type in which the sausages are smoked and dried, and the southern European sausages that are dried with or without mold present (35). Generally the sausages from southern Europe are drier than the sausages from northern Europe (semi-dry). Most fermentations are carried out at 17–24°C, although variations occur. For example, U.S. pepperoni sausages are fermented at 40°C. Table 8 shows which microorganisms are used as starter cultures for the production of sausages.

IV. YEASTS USED AS STARTER CULTURES

A. Introduction

Yeasts are involved in both spontaneous and controlled fermentations. For spontaneous fermentation processes, the yeasts are introduced by either the raw materials or via the process equipment (74–76). When yeasts are used as starter cultures, they are in general used as single cultures and may be introduced either to initiate the fermentation process or at a later stage in the fermentation to ensure optimal aroma production. Most yeast species are able to grow under both aerobic and anaerobic conditions. However, some yeast species are specifically respiratory yeasts whereas others are fermentative yeasts for which respiration is repressed even at aerobic conditions. Primarily, yeasts utilize carbohydrates as carbon sources, which are converted into alcohols and CO_2 as well as a number of secondary metabolites such as esters, organic acids, aldehydes, and ketones (77).

Yeasts involved in the fermentation of foods and beverages belong primarily to the ascomycetous yeasts. Among these, the most well described yeast species is undoubtedly *Saccharomyces cerevisiae*. This species is used worldwide for the production of bread, wine, beer, cheese and so forth and is overall the predominant yeast starter culture in use. Other important yeast species are *Saccharomyces pastorianus,* used for production of lager beer, and *Debaryomyces hansenii*, used for production of cheese and fermented meat products. A microscopic picture of *D. hansenii* cells is shown in Fig. 2. Yeast species other than the above mentioned are potential starter cultures and do often occur in high numbers during spontaneous fermentations (Table 9). The evolvements of DNA technologies and molecular typing techniques have over the recent decades influenced significantly the taxonomic position of many yeast genera and further reorganizations are expected in the future. For a current taxonomic description of yeast species, the taxonomic keys of Kurtzman and Fell (78) and Barnett et al. (79) should be consulted.

The benefits obtained by moving from spontaneous fermentations to controlled fermentations are many and, therefore, there seems to be a growing interest in the use of yeasts as purified starter cultures, not only in the control of existing fermentation processes but also in the development of new food products. In controlled fermentation, the habitat of the yeast species as well as the various functions of different yeast species should be taken into consideration as well as any probiotic property (80,81) or possible pathogenic hazard (82,83). Also, the taxonomic position of the starter cultures must be clarified and methods for typing at subspecies level introduced.

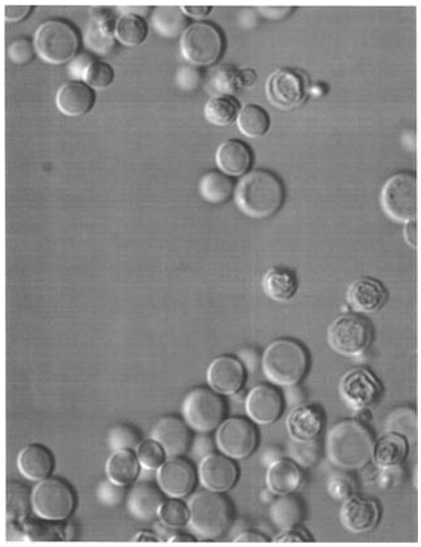

Figure 2 Cells of *Debaryomyces hansenii*. Magnification: 1000×.

Especially for brewing, wine, and baker's yeasts, efforts have been made to improve the technological properties of the strains by formation of genetically modified organisms (GMO). However, in most cases the GMOs have not been used in industrial food fermentations due to consumer resistance and statutory regulations (7).

B. Brewing Yeasts

The brewing industry has a long tradition for the use of starter cultures of brewing yeast based on single cell cultures. Worldwide, up to a thousand different brewing yeast cultures have been described. The brewing yeast strains vary in their technological properties, including aroma production, rate and degree of attenuation, flocculation, oxygen requirement, and reproduction (84). During brewing fermentation, maltose is the most dominant carbon source but sucrose, glucose, fructose, and maltotriose will also be present and utilized. Brewing yeast strains have been shown to vary in the ability to utilize maltose, and genotypic variations in the number of maltose transporter genes have been reported (85).

Two types of *Saccharomyces* yeasts are involved in beer fermentation: ale yeasts (also known as top-fermenting yeasts) and lager yeasts (also known as bottom-fermenting yeasts) (84). Ale yeasts have, since the last century, been classified as *S. cerevisiae,* whereas lager yeasts have been known under a variety of names such as *Saccharomyces carlsbergensis, S. uvarum,* and *S. cerevisiae*. The development of molecular typing techniques has revealed several genetic differences between ale and lager brewing yeasts (86–90), and according to recent classifications, lager yeasts are now considered to belong to *Saccharomyces pastorianus* (91) even though they often are still referred to as *Saccharomyces carlsbergensis* (92). However, some confusion still exists regarding the phylogenetic relationship between lager yeasts and other yeast within the genus *Saccharomyces*. It appears to be generally accepted that lager yeasts are alloploid and contain parts of two divergent genomes (93,94), one from *S. cerevisiae* and one from another *Saccharomyces* species, most likely *S. bayanus* (88,95,96)

Table 9 Examples of Yeast Species Used as Starter Cultures or Occurring Spontaneously at High Numbers in Fermented Products

Fermented foods and beverages	Yeast species[a]	Products
Beer	*Saccharomyces cerevisiae*[b] *Saccharomyces pastorianus*[b]	Ale, stout, porter, Pilsner
Wine	*Saccharomyces cerevisiae*[b] *Saccharomyces bayanus*[b] *Candida* spp. *Hanseniaspora* spp. *Kloeckera* spp. *Metschnikowia* spp. *Pichia* spp.	Red and white wine, sherry,
Indigenous fermented beverages	*Saccharomyces cerevisiae*[b] *Candida* spp. *Galactomyces geotrichum* (*G. candidum*) *Hanseniaspora uvarum* (*K. apiculata*) *Kluyveromyces africanus* *Pichia* spp. *Rhodoturula* spp. *Saccharomyces* spp. *Schizosaccharomyces pombe* *Schizosaccharomyces japonicus* *Torulaspora delbrueckii* (*C. colliculosa*)	Kafir beer, plantain beer, palm wine, sugar cane wine, sake
Distilled alcohol	*Saccharomyces cerevisiae*[b] *Schizosaccharomyces pombe*	Whisky, rum, aquavit
Bread	*Saccharomyces cerevisiae*[b] *Saccharomyces exiguous* (*C. holmii*)	Wheat bread, rye bread
Cheese	*Debaryomyces hansenii* (*C. famata*)[b] *Galactomyces geotrichum* (*G. candidum*)[b] *Saccharomyces cerevisiae*[b] *Candida zeylanoides* *Yarrowia lipolytica* (*C. lipolytica*) *Kluyveromyces lactis* (*C. spherica*) *Kluyveromyces marxianus* (*C. kefyr*)	Surface-ripened cheeses, Camembert, Gorgonzola, and other blue-veined cheeses
Fermented milk	*Galactomyces geotrichum* (*G. candidum*)[b] *Candida* spp. *Kluyveromyces marxianus* (*C. kefyr*) *Saccharomyces unisporus* *Saccharomyces* spp. *Torulaspora* spp.	Viili, kefir, indigenous sour milk
Meat products	*Debaryomyces hansenii* (*C. famata*)[b] *Candida zeylanoides* *Debaryomyces polymorphus* *Pichia guilliermondii* (*C. guilliermondii*) *Pichia membranifaciens* (*C. valida*) *Cryptococcus* spp.	Sausages, cured ham, bacon

[a] Anamorph form is given in parenthesis.
[b] Commercial starter cultures are available.

or a specific strain of *Saccharomyces monacensis* (92,97), which according to recent taxonomic keys, now also belongs to *S. pastorianus* (91).

Starter cultures of *S. cerevisiae* have also been reported to be used in the production of South African sorghum beer (98), and in addition to its use as an industrial starter culture, *S. cerevisiae* has been isolated from a variety of different indigenous spontaneously fermented beers or beer-like beverages (75).

C. Winery Yeasts and Yeasts Used for the Production of Distilled Alcohols

Traditionally, wine is produced by spontaneous fermentation and several yeast species have been reported to be involved in the fermentation. The predominant microorganisms on the grapes vary according to the grape variety, climatic conditions, soil quality, development and physical quality of the grapes, as well as the amount of fungicides applied to the vineyards. Nevertheless, the predominant yeast genera on grapes are reported to be *Kloeckera* and *Hanseniaspora,* whereas *Saccharomyces cerevisiae* is not observed or observed at only very low concentrations on healthy undamaged berries. The yeast genera associated with wine making include *Candida*, *Cryptococcus*, *Debaryomyces*, *Dekkera* (teleomorphic form of *Brettanomyces*), *Hanseniaspora* (teleomorphic form of *Kloeckera*), *Kluyveromyces*, *Metschnikowia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, and *Zygosaccharomyces*. Some of these yeast genera are thought to be essential for the wine fermentation, and others are regarded as transient organisms. Due to the low pH and high sugar content of grape juice, a natural yeast selection will take place during spontaneous wine fermentation. At the early stages of the fermentation, yeast of the genera *Candida*, *Hanseniaspora*, and *Kloeckera* will dominate, followed by species of *Metschnikowia* and *Pichia*. The latter stages of the fermentation will be dominated by alcohol-tolerant strains of *S. cerevisiae* (7).

Within the past decades there has been in wine-making an increasing interest in the use of starter cultures, and today most large-scale productions are carried out with starter cultures of primarily *S. cerevisiae*. Several different physiological variants of *S. cerevisiae* have been reported for the production of different types of wine and *S. bayanus* has been used as a starter culture, especially for wine partially produced at low temperatures (e.g., Sauternes, Tokay, Muscat, and Amarone) (99,100). Recently, genera other than *Saccharomyces* have been reported to be beneficial in the production of wine in order to enhance the taste and flavor of the wine (101,102).

Yeasts are involved in the production of several special other types of alcoholic beverages besides wine, including a large number of indigenous alcoholic beverages produced by spontaneous fermentation (9). In most cases, the yeast species responsible for the fermentation is *S. cerevisiae*. However, yeast species belonging to genera other than *Saccharomyces* have been reported—for example, for the production of sherry, where a secondary fermentation by so-called flor yeast occurs. At the early stage of the secondary fermentation, the yeast forms a surface film on the top of the wine; several yeast species, including *Pichia anomala* and *Pichia membranifaciens* yeast have been reported to be involved in this secondary fermentation. However, during the fermentation a microbial succession takes place, and *S. cerevisiae* is now believed to be the most important flor yeast for sherry production (103). Non-*Saccharomyces* yeasts have been reported to be especially involved in the early stages of indigenous, spontaneously fermented beverages (104). But at the later stages of the fermentations, *S. cerevisiae* will practically always dominate, as is the case in the brewing of sake, where isolates of *S. cerevisiae*, previously known as *Saccharomyces saké,* is used (105). For distilled alcohols, the vast majority of

modern distilleries use starter cultures of *S. cerevisiae*; exceptions are *Schizosaccharomyces pombe,* used for production of specific spirits, and lactose-fermenting yeasts, used in the production of neutral spirit from whey (106).

D. Baker's Yeast

Baker's yeast is traditionally used throughout Europe and the United States as starter culture for the production of a large variety of wheat-based breads. In Scandinavia and northern Europe, sourdough bread made from rye by back-slopping is also popular. The European tradition for production of bread seems to have spread now all over the world, including Southeast Asia and Africa (8). In all cases the dominant yeast used as starter culture is *Saccharomyces cerevisiae* Meyen ex. E.C. Hansen, as described by Vaughan-Martini and Martini (91) and Barnett et al. (79). As an alternative baker's yeast, *Saccharomyces exiguus* has been used (107).

Baker's yeast is produced as a bulk product. The global yearly production amounts to 2 million tons, and a yearly growth of approximately 4% has been reported (108). The propagation of baker' s yeast is based on a fed-batch process characterized by aerobic conditions and low carbohydrate concentrations (109). Except for the utilization of carbohydrates, optimization of baker's yeasts seems to focus on aspects quite different from those of brewing yeasts. Also, the number of commercially available baker's yeasts appears to be limited compared to brewing yeasts. Besides efficient utilization of maltose, maltotriose, and other fermentable carbohydrates present in the dough, the important technological properties of baker's yeasts appear to be biomass yield, formation of CO_2, influence on dough structure, aroma formation, and a high resistance to stress conditions such as oxidative stress, drying, freezing, and thawing (8,109–111). The latter results in a demand for high yeast concentrations of trehalose as a protective agent (112). Also, the ability of the yeast strains to adapt, from the conditions during propagation and production to the conditions in the dough, needs to be considered (109).

Types of fermented cereals other than traditional wheat bread are seen especially in areas where European-style bread is not traditionally produced, as in a great part of Africa for example. Examples of indigenous fermented cereals are products based on fermented maize, millet, and sorghum. These products are most often produced by spontaneous fermentation in which *S. cerevisiae* is the dominant yeast species, in coexistence with LAB, especially (9,50,74,113).

E. Yeasts Used for Dairy Products

For dairy products, yeasts are mainly used in cheese production but may also be involved in the production of fermented milk. Yeasts are primarily used as single-starter cultures but many products are still produced by back-slopping or spontaneous fermentation. A mixture between the used starter culture and a dominant indigenous flora is also seen in many dairy products. Yeasts are in most cases used as secondary starter cultures in order to enhance the aroma production or to facilitate the growth of other microorganisms. In general, the functions of yeasts during cheese production and their influence on the cheese quality are poorly investigated (114).

The occurrence and functions of yeasts have been especially studied for the production of surface-ripened cheeses such as Brick, Limburger, Port Salut, Taleggio, Tilsitter, Trappist, and Danish Danbo cheese. The surface smear of these cheeses is found to consist of a mixed flora comprising both yeasts and bacteria. For cheese such as Danish Danbo, the osmotolerant yeast *Debaryomyces hansenii* has been found almost exclusively (76), whereas

in other types of surface-ripened cheeses, yeasts such as *Candida zeylanoides*, *Yarrowia lipolytica*, *Kluyveromyces lactis*, and others have been found (115–117). The yeasts initiate the ripening process by degradation of lactate, thereby increasing the pH on the cheese surface and allowing the growth of a more acid-sensitive bacterial flora comprising, among others, *Brevibacterium linens* (118). It should be mentioned that the species *D. hansenii* has been divided into two varieties, *D. hansenii* var. *hansenii* and *D. hansenii* var. *fabryii* (119); apparently, the predominant variety seen in cheese production is *D. hansenii* var. *hansenii,* and isolates of this variety have been introduced as starter culture (76,118). *K. lactis* has further been found to occur in high numbers in soft cheeses such as Camembert (20). *S. cerevisiae* has also been used as a starter culture in the production of Gorgonzola, especially, but it apparently also occurs spontaneously, together with other yeasts, as an integral part of the microflora of both blue-veined cheeses and some types of soft cheeses (20,120). *Saccharomyces cerevisiae* has been reported to stimulate mycelial growth and conidia formation of *Penicillium roqueforti* as well as influencing aroma formation and having some proteolytic activity (120,121).

Even though previously considered as a mold, *Galactomyces geotrichum* (*Geotrichum candidum*) is now thought to be as a yeast species (122) and will be considered as such in the following. *G. geotrichum* is associated with milk and the dairy environment. It is known as a starter culture for several types of mold-ripened cheeses (e.g., Camembert), surface-ripened cheeses, and cheeses such as the French St. Albray. On the other hand, *G. geotrichum* has also been shown to be a potential spoilage organism. *G. geotrichum* is sometimes used in combination with other microorganisms such as *Penicillium camemberti* or *Brevibacterium linens* for the production of surface-ripened cheeses.

Even though not used as a starter culture in the dairy industry, *Yarrowia lipolytica* is often found in soft, blue-veined, and surface-ripened cheeses. *Y. lipolytica* is characterized by having a quite pronounced lipolytic and proteolytic activity (123) that might be difficult to control if used as a starter culture for cheese production. Furthermore, *Y. lipolytica* is known to produce brownish pigments in cheeses.

For fermented milk, yeasts within the genera *Candida*, *Galactomyces*, *Kluyveromyces*, *Saccharomyces*, and *Torulaspora* are generally used (124). *G. geotrichum* (*G. candidum*) is used as commercial starter culture in the production of viili, a Scandinavian fermented milk product, and *Saccharomyces unisporus* and *Kluyveromyces marxianus* (*Candida kefyr*) are used as commercial starter cultures in the production of milky kefir. *S. cerevisiae* has also been reported to be involved in the fermentation of a number of indigenous African fermented milk products known under names such as *amasi*, *nono*, and *rob* (125–127).

F. Yeasts Used for Meat Products

The use of yeasts in the fermentation of meat products is not well developed. An exception is the use of the osmotolerant yeast *D. hansenii* for the production of sausages and a few other meat products (128). *D. hansenii* is aerobic and, therefore, primarily found on the outer parts of fermented meat products; its depletion of oxygen has a color-stabilizing effect. Further, *D. hansenii* is reported to have proteolytic activity against sarcoplasmic proteins and several peptides (128) and to have lipolytic activity against pork fat (129). Several meat products, including high quality products such as Parma and Serrano hams, are still spontaneously fermented and yeasts are also involved in these types of fermentation. In addition to *D. hansenii*, *Candida zeylanoides* has been found to be involved in the ripening of Iberian dry-cured ham (130), *C. zeylanoides* and *Pichia membranifaciens* in British sausages (131), and *Cryptococcus laurentii*, *Cryptococcus humicolus*, *Debaryomyces polymorphus*, and *Pichia guilliermondii* in Portuguese cured ham and bacon (132).

V. MOLDS USED AS STARTER CULTURES

A. Introduction

In Europe, starter cultures of molds are primarily used for the production of cheese and meat, and worldwide the production of a variety of indigenous foods is based on spontaneous mold fermentations (Table 10). Molds grow only in the presence of oxygen, which limits their applications. In general, molds are known as effective producers of enzymes, and their proteolytic and lipolytic activities are often high; several molds also have extracellular glycoamylase activity. Furthermore, molds are known to produce a variety of different aroma compounds, of which the best known are alcohols and organic acids. Molds preferentially grow on carbohydrates but may also grow in protein-rich media without carbohydrates, in which they use amino acids as carbon source (133). The taxonomy of molds is primarily based on their micromorphology and growth characteristics on different media, but their production of secondary metabolites can also be used as taxonomic characters (134). Unfortunately, DNA technology and the use of molecular typing techniques have not developed as fast for molds as for yeasts, and the typing of molds used as starter cultures is still based primarily on phenotypic criteria. A taxonomic description of food-borne fungi is given by Samson et al. (135).

Table 10 Examples of Mold Species Used as Starter Cultures or Occurring Spontaneously at High Numbers in Fermented Products

Fermented foods and beverages	Mold species	Products
Cheese	*Penicillium roqueforti*[a]	Roquefort, gorgonzola,
	Penicillium camemberti[a]	Danish blue, Camembert
Meat	*Penicillium camemberti*[a]	Meat sausage, dry-cured ham
	Penicillium chrysogenum[a]	
	Penicillium nalgiovense[a]	
	Penicillium aurantiogriseum	
	Penicillium commune	
	Penicillium olsonii	
	Penicillium solitum	
	Eurotium rubrum	
Wine	*Botrytis cinerea*	Sauternes, Tokay
Indigenous fermented foods	*Aspergillus oryzae*[a]	Soy sauce, tempeh, Chinese
	Aspergillus sojae[a]	soybean paste (sufu or furu),
	Actinomucor spp.	Japanese miso and shoyu
	Mucor spp.	
	Rhizopus oligosporus	
	Rhizopus oryzea	
	Rhizopus spp.	
Fermented fish	*Aspergillus penicillioides*	Indonesian dried salted fish
	Aspergillus wentii	
	Eurotium rubrum	
Indigenous fermented beverages	*Aspergillus oryzae*	Sake, Chinese, Indian and
	Aspergillus spp.	Thai spirits, wines and beers
	Mucor spp.	
	Rhizopus spp.	

[a] Commercial starter cultures are available.

B. Molds Used for Dairy Products

The use of molds such as *Penicillium roqueforti* and *Penicillium camemberti* has a long history in the production of cheese, and their use as starter cultures goes back to the beginning of the 19th century. *P. roqueforti* is used as a secondary starter culture in the production of blue-veined cheeses, but it may also occur spontaneously in a number of other foods, and for some types of cheeses it is regarded as a contaminant. The fact that *P. roqueforti* is able to grow at high NaCl and low O_2 concentration and at a relatively high CO_2 concentration makes it suitable for the production of blue-veined cheese. During cheese maturation, *P. roqueforti* produces a number of extracellular peptidases and proteinases that are mainly responsible for the extensive proteolysis of blue-veined cheese. Large differences in proteolytic (136) and lipolytic activity (137) have been reported between different commercial strains of *P. roqueforti*. The lipolytic enzymes especially seem to be responsible for the characteristic flavor and taste of blue-veined cheeses through their production of high concentrations of methyl ketones (137). *P. camemberti* is used as a starter culture for the production of Camembert and similar types of surface-ripened cheeses, and it is highly restricted to the production of cheese and is seldom observed in other foods or in the environment (8). *P. camemberti* is able to grow on the cheese surface due to its high NaCl tolerance. On the cheese surface, it degrades lactate, resulting in an increased pH. When the lactate is depleted, the proteinases from *P. camemberti* then degrade casein, resulting in a further increase in pH and the release of ammonia. Besides its pronounced proteolytic activity, *P. camemberti* also produces lipases that are involved in aroma formation. Fig. 3 shows the mycelial growth of *P. camemberti* on a soft cheese surface.

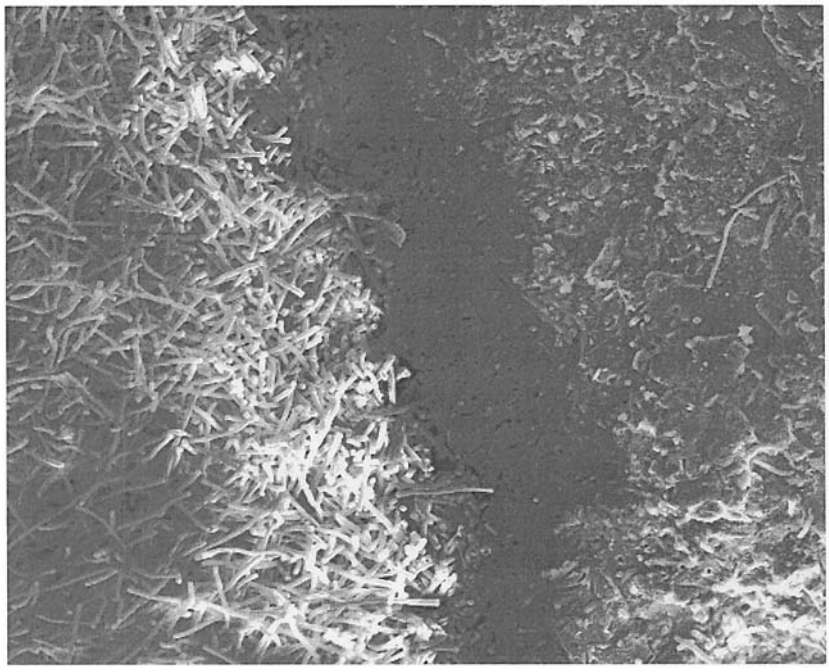

Figure 3 Growth of *Penicillium camembertii* on a soft cheese surface. Magnification: 250×.

C. Molds Used for Meat Products

The use of molds as starter cultures for fermentation of meat is mainly concentrated in such European regions as Italy, Spain, France, Germany, and Hungary. *Penicillium* spp. are mainly involved in the fermentation, and the meat products produced typically are sausages and ham. Common molds used for fermentation of meat include *Penicillium nalgiovense*, *Penicillium chrysogenum,* and *Penicillium camemberti* (8,138,139) but also other species might be used. *Penicillium commune* and *Penicillium olsonii* have been reported to be involved in the spontaneous fermentation of Spanish meat sausage (140), *Penicillium aurantiogriseum* in the fermentation of dry sausages (141), and a variety of *Penicillium* spp. including *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. commune*, *Penicillium echinulatum*, and *Penicillium expansum* have been found during spontaneous fermentation of dry-cured Iberian ham (142). For the latter, nontoxigenic strains of *P. chrysogenum* have been recommended as starter cultures (142). *Eurotium rubrum* and *Penicillium solitum* were found to be the dominant species during production of traditional Tyrolean smoked and cured ham (143). *Aspergillus* spp. are not used as starter cultures for meat fermentation but may be observed during production of cured ham, where they can grow at low water activity.

Besides influencing the appearance, molds contribute to the characteristic aroma and flavor of the product by production of extracellular proteinases and lipases (144). Also, molds inhibit the growth of unwanted microorganisms and have an antioxidative effect. Strains of both *P. chrysogenum* and *P. nalgiovense* are known penicillin producers, and the latter, at least, has been shown to be able to produce penicillin when growing on meat surfaces and to secrete it into the product (145). Therefore, starter cultures of molds must be carefully analyzed by both chemical and biological tests to ensure that they do not form either penicillin or mycotoxins in the product (139).

D. Molds in Wine Production

Molds are not used as starter culture for wine production, but the growth of *Botrytis cinerea* may be required for the production of certain types of wine. In general the growth of *B. cinerea* is unwanted, as it will rot the grapes. However, for the production of sweet white wine such as French Sauternes and Hungarian Tokay, the development of *B. cinerea* on the matured grapes is required and known as "noble" or "vulgar rot"; this results in overripening and dehydration leading to increased sugar content in the grapes. Also, the growth of *B. cinerea* adds a characteristic flavor to the wine produced from these grapes. Specific environmental conditions such as alternating dry and humid periods are required for reaching the perfect stage of maturation and the development of *B. cinerea* (146,147). Biological control of the growth of *B. cinerea* on matured grapes has been obtained by use of the yeast *Pichia membranifaciens*; its antagonistic effect against *B. cinerea* appears to be related to its secretion of exo- and endo-β-1,3-glucanases (148).

E. Molds Used for Fermentation of Indigenous Fermented Foods and Beverages

The most well known indigenous fermented products for which molds are involved in the fermentation are probably soy sauce and tempeh. Soy sauce is of Chinese or Japanese origin and is produced by an initial solid-stage mold fermentation of equal parts of cooked soybeans and roasted and crushed wheat, inoculated with conidia of *Aspergillus oryzae*

and *Aspergillus sojae*. After fermentation for 3 days, the material is placed in brine (22–25% [w/v] NaCl) for 6–8 months. During this phase, halophilic LAB and yeasts (*Zygosaccharomyces rouxii*) occur either spontaneously or are added as starter cultures. Finally, the soy sauce is harvested by pressing and is then pasteurized. Tempeh originates from Java and Indonesia and is a solid-stage mold fermentation of soaked and cooked soybeans or other leguminous seeds or cereals. After boiling and cooling, the beans are inoculated with *Rhizopus oligosporus* and *Rhizopus oryzae* and packed. After fermentation for 24–48 hr the beans are tightened together due to mycelial growth. Fresh tempeh can be eaten after cooking or frying (146). Other examples of indigenous mold-fermented foods are *sufu* or *furu*, a Chinese soybean pasta produced by *Actinomucor* spp., *Mucor* spp., or *Rhizopus* spp. (149), and Japanese *miso* and *shoyu* produced by *Aspergillus oryzae* and *Aspergillus sojae* (150). Xerophilic molds such as *Eurotium rubrum*, *Aspergillus wentii,* and *Aspergillus penicillioides* have also been reported to be involved in the fermentation of Indonesian dry salted fish (151).

Especially in Asia, species of *Aspergillus*, *Mucor,* and *Rhizopus* are used as amylase producers for fermented beverages that are based on rice or wheat (150). An example is the production of sake, in which spores of *Aspergillus oryzae* are used to break down starch to fermentable carbohydrates that are then converted to alcohol by *S. cerevisiae*. Similarly, *Aspergillus* spp., *Mucor* spp., and *Rhizopus* spp. are used in the production of Chinese, Indian, and Thai spirits, wines, and beers (152).

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4
Manufacture of Fermented Products*

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I. INTRODUCTION

The availability of fermented foods has a long history among the different cultures of the world. Acceptability of fermented foods also varies among cultures: A product highly acceptable in one may not be so regarded by consumers in another. Fermented food products are numerous. Manufacturing processes of fermented products vary considerably due to variables such as food groups, form and characteristics of final products, kind of ingredients used, and cultural diversity. It is beyond the scope of this chapter to address all the manufacturing processes used for fermented foods. Instead, this chapter is organized to address fermented food products based on food groups such as dairy, meat, cereal, soy, and vegetables. Within each food group, manufacturing processes of typical products are addressed. This chapter can be viewed as an introduction to manufacturing processes used in selected fermented food products. Readers should consult the appropriate chapters in this handbook and the references below for detailed information.

II. FERMENTED DAIRY PRODUCTS

A. Ingredients and Kinds of Products

Fermented dairy products are commonly produced in milk-producing countries and by nomadic people. These products, which are highly acceptable in these cultures, are gradually accepted by other cultures through cultural exchange. It is generally accepted that most of the fermented dairy products were first discovered and developed by nomadic peoples. The production of a fermented dairy product nowadays can be a highly sophisticated process. However, the production of another fermented dairy product can still be conducted in a fairly primitive manner in another location. Quality of a fermented dairy product varies due to the milk, microorganisms and other ingredients used in the manufacturing process. Many factors affect the gross composition of milk (1–5). The factors most significant in the processing of milk products are breed, feed, season, region, and

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Table 1 Approximate Composition of Milk

Components	Average content in milk (% w/w)	Range (% w/w)	Average content in dry matter (% w/w)
Water	87.1	85.3–88.7	
Solid-not-fat	8.9	7.9–10.0	(69)
Fat in dry matter	31	22–38	(31)
Lactose	4.6	3.8–5.3	36
Fat	4	2.5–5.5	31
Protein	3.25	2.3–4.4	25
Casein	2.6	1.7–3.5	20
Mineral substances	0.7	0.57–0.83	5.4
Organic acids	0.17	0.12–0.21	1.3
Miscellaneous	0.15		1.2

Source: Refs. 1–6.

herb health. Reviews of animal milks are available in the literature. Table 1 lists the approximate composition of milk (1–6). In industrial countries, milk composition is standardized to meet a country's requirements. However, it is understood that the requirements in one country may not be the same in another, thus the composition may vary for the same product. International agreements to standardize some products are now available. However, products produced in different locations still can vary because of microorganisms and culturing practices used in their production.

Fermented dairy products can be grossly divided into three big categories: cheeses, yogurts, and fermented liquid milks. Within each of these categories, there are subcategories. Table 2 presents examples for each of these categories (1–6).

Table 2 Kinds of Fermented Dairy Products with Examples

Kinds	Examples
Fermented liquid milks	
Lactic fermentation	Buttermilk, Acidophilus
With alcohol and lactic acid	Kefir, Kumiss
With mold and lactic acid	Viili
Concentrated	Ymer, skyr, Chakka
Yogurts	
Viscous/liquid	Yogurt
Semi-solid	Strained yogurt
Solid	Soft/hard frozen yogurt
Powder	Dried yogurt
Cheeses	
Extra hard	Parmesan, Romano, Sbrinz
Hard with eyes	Emmental, Gruyere, Swiss
Hard without eyes	Cheddar, Chester, Provolone
Semi-hard	Gouda, Edam, Caerphilly
Semi-hard, internally mold-ripened	Rouquefort, Blue, Gorgonzola
Semi-soft, surface-ripened with bacteria	Limburger, Brick, Muenster
Soft, surface mold ripened	Brie, Camembert, Neufchatel
Soft, unripened	Cream, Mozzarella, US-cottage

Source: Refs. 1–6.

In the manufacturing of fermented dairy products, various ingredients such as the milk itself, microorganism(s), coagulants, salt, sugar, vitamins, buffering salts, bleaching (decolorizing) agents, dyes (coloring agents), flavoring compounds, stabilizers, and emulsifiers may be used. The use of these ingredients in fermented liquid milks, yogurts, and natural and processed cheeses are summarized in Table 3 (1–6).

Various microorganisms such as lactic acid bacteria, yeasts, and molds are used in the manufacturing of fermented dairy products to give the various characteristics in these products. Table 4 lists some of the more common dairy microorganisms and their uses in fermented liquid dairy products, yogurts, and cheeses (7–9).

Cultures of the different microorganisms are available in various forms, such as liquid, frozen, or freeze-dried. Examples of their usage in the manufacturing of fermented dairy products are listed in Table 5 (1–6).

Because the starter cultures are available in various forms, the preparation steps of these cultures before inoculation are different. Table 6 lists some of the preparation procedures used in the industry for different forms of starter cultures (1–6).

Table 3 Ingredients for Fermented Dairy Food Production

Ingredients	Fermented liquid milk products	Yogurt	Natural cheese	Processed cheese products
Milk				
Raw	Optional	Optional	Optional	Optional
Standardized (fat and milk solids)	Preferred	Preferred	Preferred	Preferred
Milk powders	Optional	Optional	Optional	Optional
Microorganisms				
Starter bacteria	Required	Required	Required	Required
Mold	Optional	Optional	Optional	Optional
Yeast	Optional	Optional	Optional	Optional
Genetically modified microorganisms	Optional	Optional	Optional	Optional
Coagulant				
Rennet	Preferred	Preferred	Preferred	Preferred
Acid	Optional	Optional	Optional	Optional
Microbial protease(s)	Optional	Optional	Optional	Optional
Common salt (sodium chloride)	No	No	Required	Required
Sugar	Optional	Optional	No	No
Vitamins	Preferred	Preferred	Preferred	Preferred
Buffering salts (calcium chloride, hydroxide phosphates, sodium or potassium phosphates)	Optional	Optional	Optional	Optional
Bleaching (decolorizing) agents	No	No	Optional	Optional
Antimicrobial agents	Optional	Optional	No	Preferred
Dyes (coloring agents)	No	No	Optional	Optional
Flavoring compounds (fruits, spices, spice oils, fruits flavors, artificial smoke)	Optional	Optional	Optional	Optional
Stabilizers	No	Preferred	No	Preferred
Emulsifiers	Optional	Optional	No	Preferred

Source: Refs. 1–6.

Table 4 Some Common Organisms Used in Fermented Milk Products

Microorganisms	Buttermilk	Cream	Fermented milk	Yogurt	Kefir	Cheese
Bifidobacterium bifidum			X	X		X
Enterococcus durans						X
Enterococcus faecalis						X
Geotrichum candidum						X
Lactobacillus acidophilus				X		
Lactobacillus casei						X
Lactobacillus delbrueckii sub-sp. bulgaricus	X	X				X
Lactobacillus heleveticus						X
Lactobacillus kefir					X	
Lactobacillus lactis						X
Lactobacillus lactis biovar. diacetylactis		X				X
Lactobacillus lactis sub-sp. cremoris	X	X				X
Lactobacillus lactis sub-sp. lactis						X
Lactobacillus lactis var. hollandicus						X
Leuconostoc mesenteroidis sub-sp. cremoris						X
Leuconostoc mesenteroides sub-sp. dextranicum						X
Propionibacterium freudenreichii sub-sp. shermanii						X
Penicillium camemberti						X
Penicillium glaucum						X
Penicillium roqueforti						X
Streptococcus thermophilus					X	X

Source: Refs. 7–9.

Different microorganisms have different temperature requirements for their optimum growth and functions. Some fermented dairy products may require more than one microorganism to complete the manufacturing process, such as mold-ripened cheeses. These molds function best during the long ripening period and therefore have standard incubation temperatures in the refrigerated range. This is also true for some cheeses that require long ripening periods. Microorganisms requiring higher incubation temperatures are used in the production of fermented liquid milks that require only a short incubation time. Table 7 lists some of the dairy microorganisms used in some products and their incubation temperatures (7–12).

B. Cheeses

Cheeses can be classified into different categories based on their moisture, the way the milk is processed, and the types of microorganisms used for the ripening process (Table 8) (4–6).

Table 5 Dairy Starter Cultures

Physical form	Use
Liquid cultures in skim milk or whole milk (antibiotic free)	For inoculation of intermediate cultures
Liquid culture—frozen	For inoculation of intermediate cultures For inoculation into bulk cultures
Dried culture—from normal liquid culture	For inoculation of intermediate culture
Spray-dried cultures	For inoculation into bulk cultures For direct-to-vat inoculation
Frozen cultures in special media (frozen at −40°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Frozen concentrated culture (in sealed containers at −196°C)	For inoculation into bulk cultures For direct-to-vat inoculation
Single strain lypholized cultures (in foil sachets with known activity)	For inoculation into bulk cultures For direct-to-vat inoculation

Source: Refs. 1–6.

In the processing of cheese, the amount of curd used for each block of cheese to be made differs considerably (Table 9) (1,4–6,10,11). Harder cheeses have much larger blocks as compared to soft cheeses. This may be due to the ease of handling after ripening.

Cheeses are packaged in different forms to meet the consumption patterns by consumers and to some extent the way the cheese is ripened and for marketing purposes. The various packaging materials are selected to protect the cheeses in a sanitary condition, extend the shelf life, and delay the deterioration of the final products. Table 10 lists some of the requirements of cheese packaging materials (1–3,5,6,10,11,14).

All the cheeses produced have to be coagulated from acceptable milk to form the curd followed by removal of the whey. Most of the cheeses are made from standardized and pasteurized milk. Nonpasteurized milk is also used in some exceptional cases provided that the raw materials don't carry pathogens. The majority of cheeses are made from cow's milk.

Table 6 Types of Starter Cultures and Their Preparation Prior to Use

Kinds	Preparation steps	Timing
Regular starter culture	Preparation of starter culture blanks	8:00 a.m.
	Storing milk blanks	11:00 a.m.
	Activating lypholized culture powder	3:00 p.m.
	Daily mother culture preparation	3:00 p.m.
	Semi-bulk and bulk starter preparation	3:00 p.m.
Frozen culture and bulk starter application	Store frozen culture at −40°C or less Warm to 31°C and use directly	
Reconstituted milk or whey-based starter	Reconstitution	8:00 a.m.
	Heating and tempering	8:30 a.m.
	Inoculating and incubating	10:00 a.m.
Bulk starter from ultrafiltered milk	Untrafiltration	1:00 p.m.
	Heating and tempering	3:30 p.m.
	Inoculating and incubating	5:00 p.m.

Source: Refs. 1–6.

Table 7 Temperature Requirements and Acid Production for Some Dairy Microbes

Microorganisms	Product group	Standard temperature for incubation, °C	General maximum titratable acidity produced in milk, %
Bacteria			
Bifidobacterium bifidum	1	36–38	0.9–1.0
Lactobacillus acidophilus	1	38–44	1.2–2.0
Lactobacillus delbrueckii subsp. *bulgarius*	1	43–47	2.0–4.0
Lactobacillus lactis subsp. *cremoris*	2	22	0.9–1.0
Lactobacillus subsp. *lactis*	2	22	0.9–1.0
Leuconostoc mesenteroides subsp. *cremoris*	2	20	0.1–0.3
Streptococcus durans	2	31	0.9–1.1
Streptococcus thermophilus	2	38–44	0.9–1.1
Molds			
Penicillium roqueforti	3	11–16	NA
Penicillium camemberti	3	10–22	NA

Product group: 1 = yogurt, 2 = fermented liquid milk, 3 = cheese.
Source: Refs. 7–13.

Table 8 Classification of Cheese According to Moisture Content, Scald Temperature and Method of Ripening

Hard cheese (moisture 20–42%; fat-in-dry-matter, 32–50% min.)			
Low scald, lactic starter	Medium scald, lactic starter	High scald, propionic eyes	Plastic curd, lactic starter, or propionic eyes
Gouda	Cheddar	Parmesan	Provolone
Cheshire	Svecia	Beaufort	Mozzarella

Semi-hard cheese (moisture 45–55%; fat-in-dry matter, 40–50%, min.)		
Lactic starter	Smear coat	Blue-veined mold
St. Paul	Limburg	Roquefort
Lanchester	Muenster	Danablue

Soft cheese (moisture >55%; fat in dry matter, 4–51%, min.)				
Acid coagulated	Smear coat or surface mold	Surface mold	Normal lactic starter	Unripened fresh
Cottage cheese (USA)	Brie	Camembert	Quarg	Cottage (UK)
Quesco blanco	Bel Paese	Neufchatel	Petit Suisse	York

Source: Refs. 4–6.

Table 9 Approximate Weight of Cheese Varieties

Cheese variety	Approimate weight (kg)
Hard to semi-hard or semi-soft	
Wensleydale	3–5
Caerphilly	3–6
White Stilton	4–8
Single Gloucester	10–12
Leichester	13–18
Derby	14–16
Sage Derby	14–16
Cheddar	18–28
Cheshire	20–22
Dunlap	20–27
Double Gloucester	22–28
Lancashire	22
Internally mold-ripened (blue-veined) cheese	
Blue Wensleydale	3–5
Blue Vinney	5–7
Blue Stilton	6–8
Blue Cheshire	10–20
Soft cheese	
Colwich	0.25–0.50
Cambridge	0.25–1.0
Melbury	2.5

Source: Refs. 1, 4–6, 10–11, and 13.

Table 10 Requirements of Cheese Packaging Materials

Low permeability to oxygen, carbon dioxide, and water vapor
Strength and thickness of film
Stability under cold or warm conditions
Stability to fats and lactic acid
Resistance to light, especially ultraviolet
Ease of application, stiffness, elasticity
Ability to seal and accept adhesives
Laminated films to retain laminated
Low shrinkage or aging unless shrinkage is a requisite
Ability to take printed matter
Should not impart odors to the cheese
Suitability for mechanization of packaging
Hygienic considerations in storage and use
Cost-effectiveness as a protective wrapping

Source: Refs. 1–3, 5, 6, 10, 11, and 14.

Table 11 Basic Cheese-Making Steps

Standardization of cheese milks
Homogenization of cheese milks
Heat treatment or pasteurization of cheese milks
Starter addition
Addition of color and additives
Coagulation/curdling
Cutting the coagulum/curd
Stirring and scalding
Washing of curd cheese
Salting of cheese
Pressing of cheese
Coating, bandaging, and wrapping of cheese
Ripening
Retail packaging
Storage

Source: Refs. 1–11, and 14.

Milks from other animals are also used for specialty products. The coagulation process is conducted through the addition of the coagulant (rennin or chymosin) and incubation of appropriate lactic acid bacteria in milk to produce enough acid and appropriate pH for curdling of the milk. After the casein is recovered, it is salted and subject to fermentation with or without inoculation with other microorganisms to produce the desirable characteristics of the various cheeses. Due the variations in the different manufacturing steps, the result is a wide variety of cheeses with various characteristics. Table 11 summarizes the basic steps in a cheese manufacturing process (1–11,14). Table 12 summaries the ripening conditions for various cheeses. Selected examples are then introduced below to provide an overview of the complexity of cheese manufacturing (1–11,14,15).

1. Cottage Cheese Manufacturing

Cottage cheese is a product with very mild fermentation treatment. It is produced by incubating (fermenting) the standardized and pasteurized milk with the starter lactic acid bacteria to produce enough acid and appropriate pH for the curdling of milk. The curd is then recovered and washed, followed by optional salting and creaming. The product is

Table 12 Cheese-Ripening Conditions

Types of cheese	Storage period (days)	Temperature (°C)	Relative humidity (%)
Soft	12–30	10–14	90–95
Mold-ripened	15–60	4–12	85–95
Cooked (e.g., Emmental)			
Cold room	7–25	10–15	80–85
Warm room	25–60	18–25	80–85
Hard (e.g., Cheddar)	45–360	5–12	87–95

Source: Refs. 1–11, 14.

then packed and ready for marketing. No further ripening is required for this product. This is different from most fermented cheeses that require a ripening process. Table 13 lists the various steps involved in the production of cottage cheese (1–3,5,6,10,14).

2. Cheddar Cheese Manufacturing

Cheddar cheese is a common hard cheese without eyes used in the fast food industry and in households. Its production process is characterized by a requirement for milling and cheddaring of the curd. This cheese can be ripened with a wax rind or rindless (sealed under vacuum in plastic bags.) It is also categorized into regular, mild, or sharp based on the aging period (45–360 days). The longer the aging period, the sharper the flavor. It is packaged as a large block or in slices. Table 14 lists the basic steps in the manufacturing of Cheddar cheese (1–3,5,6,10,14).

3. Swiss Cheese Manufacturing

Swiss cheese is also a common cheese used in the fast food industry and in households. It is characterized by having irregular eyes inside the cheese. These eyes are produced by

Table 13 Basic Steps in Cottage Cheese Making

Standardization of skim milk
Pasteurization of milk with standard procedure and cooling to 32°C
Inoculation of active lactic starter, addition of rennet, and setting of curd
  Rennet addition—at 2 ml single strength (prediluted, 1:40) per 1000 kg milk within 30 min of starter addition

Type of activity	Short set	Medium set	Long set
Starter concentration	5%	3%	0.5%
Temperature of milk set	32°C	27°C	22°C
Setting to cutting	5 hr	8 hr	14–16 hr

  Final pH and whey titratable acidity—4.6, 0.52% whey titratable acidity, restenosisspectively
Cutting of curd with 1.3, 1.6, or 1.9 cm wire cheese knife
Cooking of curd:
  Let curd cubes stand for 15–30 min and cook to 51–54°C with 1.7°C per 10 min
  Roll the curds gently every 10 min after initial 15–30 min wait
  Test curd firmness and hold 10–30 min longer to obtain proper firmness
Washing of curd
  First wash with 29°C water temperature
  Second wash with 16°C water temperature
  Third wash with 4°C water temperature
Gravitational draining of washed curd for about 2.5 hr
Salting and creaming at 152 kg creaming mixture per 454 kg with final 0.5–0.75% salt content and 4% fat content (varies with products and optional)
Packaging in containers
Storage at refrigerated temperature

Source: Refs. 1–3, 5, 6, 10, and 14.

Table 14 Basic Steps in the Making of Cheddar Cheese

Standardization of cheese milk
Homogenization of milk
Pasteurization and additional heating of milk
Cooling of milk to 31°C
Inoculation of milk with lactic starter (0.5–2% active mesophilic lactic starter)
Addition of rennet or other protease(s)—198 ml single strength (1:15,000) rennet per 1,000 kg milk. Dilute the measured rennet 1:40 before use. Agitate at medium speed.
Setting the milk to proper acidity—25 min
Cutting the curd using 0.64 cm or wider wire knife. Stir for 5 min at slow speed
Cooking the curd at 38°C for 30 min with 1°C every 5 min increment. Maintain temperature for another 4–5 min and agitate periodically at medium speed.
Draining the curd at 38°C
Cheddaring the curd at pH 5.2 to 5.3
Milling the curd slabs
Salting the curd at 2.3 to 3.5 kg salt per 100 kg curd in 3 portions in 30 min
Waxed cheddar cheese
Hooping and pressing at 172 kPa for 30–60 sec then 172–344 kPa overnight
Drying the cheese at 13°C at 70% R.H. for 2–3 days
Paraffin whole cheese at 118°C for 6 sec
Rindless cheddar cheese
Pressing at 276 kPa for 6–18 hours
Pre-press for 1 min followed by 45 min under 686 mm vacuum
Remove and press at 345 kPa for 60 min
Remove and vacuum seal in bags with hot water shrinkage at 93°C for 2 sec
Ripening at 85% R.H. at 4°C for 60 days or longer, up to 9–12 months, or at 3°C for 2 months then 10°C for 4–7 months, up to 6–9 months

Source: Refs. 1–3, 5, 6, 10, and 14.

Propionibacterium freudenreichii sub-sp. *shermanii* that produces gases trapped inside the block of cheese during fermentation and ripening. A cheese with eyes like Swiss cheese has become the icon for cheese in graphics. Swiss cheese is also characterized by its propionic acid odor. The salting process for Swiss cheese utilizes both the dry and brine salting processes. Like Cheddar cheese, Swiss can be categorized into regular, mild, and sharp depending on the length of the curing process. Table 15 lists the basic steps in the manufacture of Swiss cheese (1–3,5,6,10,14).

4. Blue Cheese

Blue cheese is characterized by its strong flavor and blue mold filaments from *Penicillium roqueforti* inside the cheese. It is commonly consumed as such or made into a salad dressing. In the manufacturing of blue cheese, like the Swiss cheese, salting is accomplished by the application of dry salting and brining processes. It is characterized by a bleaching step of the cream to show off the blue mold filament with a lighter background and needling of the block of curd for spreading of the blue mold filaments. It also has a soft and crumbly texture due to the needling process, and a gravity-draining procedure of the curd. The curing period of 2–4 months is shorter than that of the hard cheeses. Its shelf life of 2 months is also shorter than its harder counterparts. Table 16 lists the basic steps in the manufacture of blue cheese (1–3,5,6,10,14).

Table 15 Basic Steps in Swiss Cheese Making

Standardization of cheese milk to 3% milk fat—Treatment with H_2O_2-catalase optional
Pasteurization of the milk
Inoculation with starters
Streptococcus thermophilus, 330 ml per 1000 kg milk
Lactobacillus delbruechii subsp. bulgaricus, 330 ml per 1000 kg milk
Propionibacterium freudenreichii sub-sp. shermanii, 55 ml per 1000 kg milk
Addition of rennet, 10–20 min after incoculation
154 ml single-strength (1:15,000) rennet extract per 1000 kg milk, prediluted 1:40 with tap water before addition
Stir for 3 minutes
Setting (coagulation) of milk in 25–30 min
Cutting the curd with 0.64 wire knife and letting curd stand undisturbed for 5 min and stirring at medium speed for 40 min
Cooking the curd slowly to 50–53°C for about 30 min and stirring at medium speed; then turning off steam and continued stirring for 30–60 min with pH reaching 6.3 to 6.4
Dripping the curd for 30 min
Pressing the curd with preliminary pressing then at 69 kPa overnight
First salting—in 23% salt brine for 2–3 days at 10°C.
Second salting—at 10–16°C, 90% R.H. for 10–14 days by wiping the cheese surface from brine soaking followed by sprinkling of salt over cheese surface daily
Third salting—at 20–24°C, 80–85% R.H. Wash cheese surface with salt water and sprinkle with dry salt 2–3 times weekly for 2–3 weeks
Rinded block Swiss cheese
Curing—at 7°C or lower (U.S.) or 10–25°C (Europe) for 4–12 months
Packing in container and storing at cool temperature
Rindless block Swiss cheese
Wrapping the block or vacuum-pack the blocks
Curing stacked cheese at 3–4°C for 3–6 weeks
Storing at cool temperature

Source: Refs. 1–3, 5, 6, 10, and 14.

5. American-Style Camembert Cheese

American-style Camembert cheese is categorized as a soft cheese. It is characterized by having a shell of mold filament on the surface produced by *Penicillium camemberti*. Brie cheese is a similar product. Addition of annatto color is optional. Like blue cheese, it is gravity drained; therefore, it provides a soft and smooth texture. This cheese is surface salted and has a total curing period of 3 weeks before distribution. It is usually cut into wedges and wrapped individually for direct consumption. Table 17 lists the basic steps in the manufacture of American-style Camembert cheese (1–3,5,6,10,14).

6. Feta Cheese Manufacturing

Feta cheese is a common cheese in the Mediterranean countries. It is a soft cheese and is characterized by its brine curing (maturation) process that is not common in cheese making. Instead, it has similarity to the manufacture of sufu (Chinese fermented tofu, see below in this chapter and also in Chapter 31). Like the other soft cheeses, the curing period is only 2–3 months. Table 18 lists the basic steps in the manufacture of Feta cheese (15).

Table 16 Basic Steps in Blue Cheese Making

Milk preparation
Separation of cream and skim milk
Pasteurize skim milk by HTST, cool to 30°C
Bleach cream with benzoyl peroxide (optional) and heat to 63°C for 30 sec
Homogenize hot cream at 6–9 mPa and then 3.5 mPa, cool and mix with pasteurized skim milk
Inoculation with 0.5% active lactic starter to milk at 30°C. Let stand for 1 hr
Addition of rennet:
158 ml single-strength (prediluted 1:40) per 1000 kg milk and mix well
Coagulation or setting in 30 min
Cutting curd with 1.6 cm standard wire knife
Cooking of curd at 30°C followed by 5 min standing and then agitation every 5 min for 1 hr. Whey should have 0.11 to 0.14 titratable acidity
Draining of whey by gravity for 15 min
Inoculation with *Penicillium roqueforti* spores—2 kg coarse salt and 28 g *P. roqueforti* spore powder per 100 kg curd followed by thorough mixing. Addition of food-grade lipase optional.
First salting by dipping the curd in 23% brine for 15 min followed by pressing or molding at 22°C with turning every 15 min for 2 hr and every 90 min for rest of day
Second salting on cheese surface every day for 5 days at 16°C, 85% R.H.
Final dry salting or brine salting in 23% brine for 24–48 hr. Final salt concentration about 4%
Incubation for 6 days at 16°C, R.H.
Waxing and needling air holes, or vacuum pack and needling air holes
Mold filament development in air holes at 16°C for 6–8 days
Curing at 11°C 95% R.H. for 60–120 days
Cleaning and storing
Strip off the wax or vacuum-packaging bag
Clean cheese, dry and repack in aluminum foil or vacuum-packaging bags
Store at 2°C
Product shelf life—2 months

Source: Refs. 1–3, 5, 6, 10, and 14.

C. Yogurt

Yogurt can be considered a curdled milk product. Plain yogurt is yogurt without addition of other flavor and stabilizer or coagulant. Its acceptance is limited to those few who really enjoy eating it. With the development of technology, other forms of yogurt are now available, such as flavored and sweetened yogurt, stirred yogurt, yogurt drinks, and frozen yogurt. Its popularity varies by location. It is considered a health food when active or live cultures are added to the final product. Table 19 lists the basic steps involved in the manufacture of yogurt. Table 3 should also be consulted for reference to other ingredients (16,17).

Most commercially produced yogurt and its products contain sweeteners, stabilizers, or gums (Table 20), fruit pieces, natural and synthetic flavors (Table 21), and coloring compounds (Table 22) (10,17).

Different countries also have different standards on the percent of fat and percent of solids-not-fat (SNF) contents in their yogurt products (Table 23) (10,17).

The different variables described above, make the situation complicated. The term "yogurt" in one country may not have the same meaning in another country. It also makes it difficult for international trade. Consensus or agreement among countries, and proper labeling, are needed to identify the products properly.

Table 17 Basic Steps in American-Style Camembert Cheese

Standardization of milk
Homogenization of milk
Pasteurization of milk at 72°C for 6 sec
Cooling milk to 32°C
Inoculation with 2% active lactic starter followed by 15–30 min acid ripening to 0.22% titratable acidity
Addition of annatto color at 15.4 ml per 1000 kg milk (optional)
Addition of rennet—220 ml single strength (prediluted 1:40) per 1000 ml followed by mixing for 3 min and standing for 45 min
Cutting of curd with 1.6 cm standard wire knife
Cooking of curd at 32°C for 15 min with medium speed stirring
Draining of curd at 22°C for 6 hr with occasional turning
Inoculation with *Penicillium camemberti* spores through spray gun on both side of cheese once
Pressing and molding curd by pressing for 5–6 hr at 22°C without any weight on surface
Surface salting of cheese and letting stand for about 9 hr
Curing at 10°C, 95% R.H. for 5 days undisturbed, then turned once and continue curing for 14 days
Packaging, storage, and distribution
Wrap cheese and store at 10°C, 95–98% R.H. for another 7 days
Move to cold room at 4°C and cut into wedges, if required, and rewrap
Distribute immediately

Source: Refs. 1 2 3, 5, 6, 10, and 14.

D. Fermented Liquid Milks

In milk-producing countries, it is common to have fermented milk products. These products are first discovered or developed by accident. Later, the process is modified for commercial production. Fermented liquid milks are similar to plain yogurt drinks. It is basically milk that has gone through an acid and/or alcoholic fermentation. The final product is maintained in the liquid form, as compared to yogurt that is mostly in the soft-

Table 18 Basic Steps in Feta Cheese Making

Standardization of milk with 5% fat, enzyme treated and decolorized
Homogenization of milk
Pasteurization by standard procedure and cooling to 32°C
Inoculation with 2% active lactic starter as cheddar cheese followed by 1 hr ripening
Addition of rennet at 198 ml single strength (prediluted, 1:40) per 1000 kg milk followed by 30–40 min setting
Cutting of the curd with 1.6 cm standard wire knife followed by 15–20 standing
Dripping of curd for 18–20 hr at 12–18 kg on 2000 sq. cm with pH and titratable acidity developed to 4.6 and 0.55%, respectively
Preparation of cheese blocks of 13×13×10 cm each
Salting 23% salt brine for 1 day at 10°C
Canning and boxing cheese blocks in 14% salt brine (sealed container)
Curing for 2–3 months at 10°C
Soaking cured cheese in skim milk for 1–2 days before consumption to reduce salt
Yield—15 kg/100 kg of 5% fat milk

Source: Ref. 15.

Table 19 Basic Steps in the Production of Yogurt

Standardization of liquid milk
Homogenization of liquid milk
Heat treatment or pasteurization of liquid milk at 90°C for 5 min or equivalent
Cooling of pasteurized milk to 1–2°C above inoculation temperature
Addition of starter (inoculation), 1–3% operational culture
Addition of flavor, sweetener, gums, and/or color (optional)
Incubation at 40–45°C for 2.5–3.0 hr for standard cultures
Breaking of curd (optional)
Cooling to 15–20°C in 1–1.5 hr
Addition of live culture (optional)
Packaging
Storage at ≤10°C

Source: Refs. 16 and 17.

Table 20 Some Common Gums That Could Be Used in Yogurt Manufacturing

Kind	Name of gum
Natural	Agar
	Alginates
	Carageenan
	Carob gum
	Cornstarch
	Casein
	Fulcelleran
	Gelatin
	Gum arabic
	Guar gum
	Karaya gum
	Pectins
	Soy protein
	Tragacanth gum
	Wheat starch
Modified gums	Cellulose derivatives
	Dextran
	Low-methoxy pectin
	Modified starches
	Pregelatinized starches
	Propylene glycole alginate
	Xanthin
Synthetic gums	Polyethylene derivatives
	Polyvinyl derivatives

Source: Refs. 10 and 17.

Table 21 Some Common Yogurt Flavors

Retail flavor	Natural characteristic-impact compound	Synthetic flavoring compound available
Apricot	NA	γ-Undecalactone
Banana	3-Methylbutyl acetate	NA
Bilberry	NA	NA
Blackcurrant	NA	*trans*- and *cis*-*p*-Methane-8-thiol-3-one
Grape, Concord	Methyl antranilate	NA
Lemon	Citral	15 compounds
Peach	g-Decalactone	γ-Undecalactone
Pineapple	NA	Allyl hexanoate
Rasberry	1-*p*-Hydroxyphenyl-3-butanone	NA
Strawberry	NA	Ethyl-3-methyl-3-phenylglycidate

Source: Refs. 10 and 17.

gel form. There are different fermented liquid milks available, and only sour milk, kefir, and acidophilus milk are discussed below. Readers should also refer to other chapters in this book on related products.

1. Sour Milk

Table 24 presents the basic steps in the manufacturing of the most basic fermented liquid milk, sour milk. The milk is standardized, pasteurized, inoculated, incubated, homogenized, and packaged. It is a very straightforward procedure as compared to the other two products, kefir and acidophilus milk (1–9).

2. Kefir

Kefir is a fermented liquid milk product characterized by its content of a small amount of alcohol, and its inoculant, the kefir grains. It is a common product in Eastern European

Table 22 Permitted Yogurt Colorings

Name of color	Maximum level (mg/kg)
Intigotine	6
Brilliant black PN	12
Sunset yellow FCF	12
Tartrazine	18
Conchineal	20
Carminic acid	20
Erythrosine	27
Red 2G	30
Ponceau	48
Caramel	150
Brilliant blue FCF	200

Source: Refs. 10 and 17.

Table 23 Existing or Proposed Standards for Commercial Yogurt Composition [% Fat and % Solid-Not-Fat(SNF)] in Selected Countries

	% Fat			
Country	Low	Medium	Normal	% SNF
Australia	NA	0.5–1.5	3	NA
France	0.5	NA	3	NA
Italy	1	NA	3	NA
Netherlands	1	NA	3	NA
New Zealand	0.3	NA	3.2	NA
United Kingdom	0.3	1.0–2.0	3.5	8.5
United States	0.5–1.0	2	3.25	8.5
West Germany	0.5	1.5–1.8	3.5	8.25–8.5
FAO/WHO	0.5	0.5–3.0	3	8.2
Range	0.3–1.0	0.5–3.0	3–3.5	8.2–8.5

Source: Refs. 10 and 17.

countries and is considered to have health benefits. Among all the fermented dairy products, only this and similar product contain small amount of alcohol. Also, among all the fermented dairy products, pure cultures of bacteria, yeasts, and/or mold are used, but in kefir, the kefir grains are used and recycled. Kefir grains are masses of bacteria, yeasts, polysaccharides, and other products of bacterial metabolism, together with curds of milk protein. Production of kefir is a two-step process: first, the production of mother kefir, followed by the production of the kefir drink. Table 25 lists the basic steps in kefir manufacturing (1–9,18).

3. Acidophilus Milk

Acidophilus milk is considered to have probiotic benefits. Like yogurt, it is advertised as having live cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. These live cultures are claimed to provide the benefit of maintaining a healthy intestinal microflora. Traditional acidophilus milk has a considerable amount of lactic acid and is considered too sour for consumers in some locations. Therefore, a small amount of sugar is added to the final product to make it more palatable. This later product is called sweet acidophilus milk. Table 26 lists the basic steps in the manufacture of acidophilus milk (1–9).

Table 24 Basic Steps in Sour Milk Processing

Standardization of milk
Heating of milk to 85–95°C followed by homogenization
Cooling of milk to 19–25°C and transfer of milk to fermentation tank
Addition of 1–2% start culture (inoculation)
Shock-free fermentation to pH 4.65–4.55
Homogenization of gel
Cooling to 4–6°C
Filling into bottles, jars, or one-way packs or wholesale packs

Source: Refs. 1–9.

Table 25 Basic Steps in Kefir Processing

Preparation of "mother" kefir
Standardization of milk for preparation of mother kefir
Pasteurize milk at 90–95°C for 15 min and cool to 18–22°C
Spread kefir grains at the bottom of a container (5–10 cm thick) and add pasteurized milk (20–30 times the amount of kefir grains)
Ferment for 18–24 hr with mixing 2–3 times. Kefir grains float to the surface
Filter out the kefir grains with a fine sieve; wash the grains with water, and save for the next fermentation
Save the fermented milk for the next-step inoculation
Preparation of drinkable kefir
Blend fermented milk from above with 8–10 times fresh, pasteurized, and untreated milk
Fill into bottles, closed and fermented for 1–3 days at 18–22°C
[Another option is to mix the fermented milk with fresh milk at 1–5%; ferment at 20–25°C for 12–15 hr until pH 4.4–4.5 followed by ripening in storage tanks 1–3 days at 10°C. Product is not as traditional but acceptable.]
Cool to refrigerated temperature
Store and distribute

Source: Refs. 1–9, 18.

Table 26 Basic Steps for Sweet Acidophilus Milk Processing

Procedure #1
Standardization of milk
Heating milk to 95°C for 60 min, cooling to 37°C, and holding for 3–4 hr, reheating to 95°C for 10–15 min, cooling to 37°C
Inoculation with 2–5% bulk starter
Incubation for up to 24 hr or to 1% lactic acid
Cooling to 5°C
Packing and distribution
Procedure #2
Standardization of milk
Homogenization of milk at 14.5 MPa
Heating to 95°C for 60 min
Cooling to 37°C
Inoculation with Direct Vat Inoculation (DVI) starter
Incubation for 12–16 hr or to about 0.65% lactic acid
Ultra High Temperature (UHT) of 140–145°C for 2–3 sec to eliminate undesirable contaminants
Cooling to 10°C or lower
Packaging and distribution

Source: Refs. 1–9.

III. MEAT PRODUCTS

A. Ingredients and Types

Fermented meat products such as ham and sausages have been available in different cultures for centuries. It is interesting to learn that the ways these products are produced are basically very similar in different cultures. Besides the meat, nitrite and salt, and sugar (optional), pure cultures are sometimes used, especially in fermented sausages. Microorganisms not only provide the characteristic flavor for the products, but the lactic acid bacteria also produce lactic and other acids that can lower the pH of the products. Pure cultures are sometimes used in hams to lower the pH for the benefit of inhibition of growth of *Clostridium botulinum*. The raw meat for ham manufacturing is basically a large chunk of meat. It is difficult for the microorganisms to penetrate into the center, unless they are injected into the interior. The microbial growth is mainly on the surface and their enzymes are gradually diffused into the center. In comparison, for sausages, the cultures, if used, are mixed with the ingredients at the beginning and the fermentation is carried out without difficulty. Besides, sausages are much smaller than hams. Table 27 lists some of the ingredients used in the manufacture of hams and sausages (19–27).

B. Hams

Hams, as indicated earlier, are made from large chunks of meat. Western cultures manufacture ham using either the dry cure and/or brine cure processes, sometimes followed by a smoking process. Tables 28 and 29 list the basic steps involved with the dry cure and brine cure of hams, respectively. These two processes are similar except for the salting step (20,21).

Table 27 Raw Ingredients for Fermented Meat Products

Ingredient	Ham	Sausage
Meat		
Pork	Yes	Yes
Beef	No	Optional
Casing	No	Yes
Salt	Yes	Yes
Sugar	Optional	Optional
Starter microorganisms	Optional	Optional
Lactobacillus sakei, *L. curvatus*, *L. plantarum*, *L. pentosus*, *L. pentoaceus*		
Pediococcus pentosaceus, *P. acidilactic*		
Staphylococcus xylosus, *S. carnosus*		
Kocuria varians		
Debaryomyces hansenit		
Candida famata		
Penicillium nagiovense, *P. chrysogenum*		
Spices	Optional	Optional
Other flavoring compounds	Optional	Optional
Moisture retention salts	Optional	Optional
Preservatives	No	No

Source: Refs. 19–27.

Table 28 Basic Steps in Dry-Cured Ham Processing

Preparation of pork for dry curing
Mixing of the proper ratio of ingredients [salt, sugar, nitrite and inocula (optional)]
Rubbing of the curing mixture to the meat
Stacking of the green ham for initial dry curing at 36–40°C
Re-rubbing of the green ham and stacking for additional curing at 36–40°C
[The ham should be left in the cure for the equivalent of 3 days per pound of meat.]
Soaking of the cured ham for 2–3 hours followed by thorough scrubbing
Placing green ham in tight-fitting stockinette and hanging in smokehouse to dry overnight
Smoking at about 60 or 80°C with 60% R.H. for 12–36 hr
Cooling
Vacuum packaging and cool storage

Source: Refs. 20 and 21.

Chinese hams are basically manufactured using a dry curing process. Procedures differ slightly depending on where the hams are made. The most famous Chinese ham is the Jinghua ham, made in the central China region. Yunan ham in the southern China region also has a good reputation. In the old days, without refrigeration facilities during processing, transportation, and storage, it is believed that the ham completed its aging process during the transportation and storage stages. Nowadays, with controlled temperature and relative humidity rooms, hams are produced under controlled conditions. Table 30 lists the current process used in China for Jinghua ham (19,27).

C. Sausages

Many European-type sausages are manufactured using a fermentation process. These sausages have their own characteristic flavors due to the formulations and curing processes used. It is not the intent of this chapter to list the various formulations. Readers should consult the references in this chapter, the chapter on sausages in this handbook, and other references available elsewhere. Commercial inocula are available. Bacteria and some yeasts grow inside the sausage during the ripening period, producing the characteristic flavor.

Table 29 Basic Steps in Brine-Cured Ham Processing

Preparation of pork for brine curing
Mixing of the proper ratio of ingredients (salt, sugar, and nitrite with inocula optional; five gallons of brine for 100 lb meat)
Soaking of the meat in the prepared brine, or stitch pumping of the brine into the meat (10% of the original weight of the meat) followed by soaking in the brine for 3–7 days vacuum tumbling or massaging (optional)
Removal of the meat from the cover brine and washing
Placing green ham in tight-fitting stockinette and hanging in smokehouse to dry overnight
Smoking at about 60 or 80°C with 60% R.H. for 12–36 hr
Cooling
Vacuum packaging and cool storage

Source: Refs. 20 and 21.

Table 30 Basic Steps in Chinese Jinghua Ham Processing

Selection of pork hind leg, 5–7.5 kg
Trimming
Salting, 7–8 kg salt per 10 kg ham
Stacking and overhauling at 0–10°C for 33–40 days
Washing with cold water and brush
Drying in the sun for 5–6 days
Fermentation (curing) for 2–3 months at 0–10°C with harmless green mold developing on surface
Brushing off the mold and trimming
Aging for 3–4 months, maximum 9 months; alternate aging process in temperature-programmable room with 60% for 1–2 months
Grading
(Yield: about 55–60%)
Packaging and distribution

Source: Refs. 19 and 27.

Molds can grow on the surface during storage if they are not properly packaged and stored in the refrigerator. Because these sausages are not sterilized, fermentation is an ongoing process, with the aged sausages carrying a stronger flavor. Table 31 lists the basic steps in the manufacture of dry fermented sausages (22–24,26).

IV. FERMENTED CEREAL PRODUCTS (BREADS AND RELATED PRODUCTS)

A. Products and Ingredients

In wheat-producing countries or areas, baked yeast bread is a major staple in people's diet. This is common in the major developed countries. Elsewhere, other forms of bread may be the major staple in meals. Baked bread may come in different forms, such as regular yeast breads, flat breads, and specialty breads. Nowadays, even retarded (chilled or frozen)

Table 31 Basic Steps in Dry (Fermented) Sausage Processing

Selection of meat for processing
Chopping and mixing of chopped meat with spices, seasonings and inocula at temperature about 10°C
Stuffing the mixture in suitable casings
Linking
Curing or drying for 1–3 months in rooms with temperature, relative humidity, and air circulation regulated according to the type of sausages being produced
Packaging and cool storage

Source: Refs. 22–24, 26.

doughs are available to meet consumer preference for product similar to home-cooked foods. For countries or areas with less accessible energy, other forms of bread such as steamed bread and boiled breads are available. Fried breads are consumed mainly as breakfast or snack items. Table 32 lists examples of different types of breads (28–33).

There are different types of wheat available nowadays through centuries of breeding selections to meet production environments in various regions with diversified climatic conditions. Wheat used for making bread is hard wheat, soft wheat, or a combination of both to meet product specifications. Wheat kernels are milled with removal of the bran and germ and further processed into wheat flour. Traditionally, this flour is the major ingredient for baking bread. For some health-concerned consumers, whole wheat flour is the choice flour used in making bread nowadays. Wheat bran is also added to increase fiber content of the product. Table 33 lists the proximate composition of wheat and some common wheat products (28–30).

In the manufacture of various wheat-based breads and related products, the major ingredients are wheat flour, yeast, sourdough bacteria (optional), salt, and water. Other ingredients vary considerably with the types of product produced. They may be grossly classified as optional ingredients, additives, and processing aids. Each country has its own regulations and requirements. Table 34 lists basic ingredients, optional ingredients, additives, and processing aids used in the manufacturing of bread and related products (28–30).

B. Regular Bread

Table 35 lists the basic steps in bread manufacturing (28–30).

There are three basic processes in commercial bread making: straight dough process, sponge-and-dough process, and continuous-baking process. The choice of which process to use is up to the manufacturer and the equipment available in the baking plant. Table 36 lists the basic steps in the different processes. The major difference is in the way the dough is prepared and handled (28–30).

Because the dough may be prepared in various ways, the amounts of ingredients used also differ accordingly. Table 37 lists two formulations comparing the differences due to dough preparation processes (28–30).

Table 32 Types of Bread and Related Products

Type	Examples
Baked breads	
Regular yeast breads	Bread (white, whole wheat, or multigrain)
Flat (layered) breads	Pocket bread, croissants
Specialty breads	Sourdough bread, rye bread, hamburger bun, part-baked bread, Danish pastry, stuffed bun
Chilled or frozen doughs	Ready-to-baked doughs, retarded pizza doughs, frozen proved dough
Steamed breads	Chinese steamed bread (mantou), steamed stuffed bun
Fried breads	Doughnuts
Boiled breads	Pretzels

Source: Refs. 28–33.

Table 33 Composition of Wheat, Flour, and Germ

Material	Mositure %	Protein %	Fat %	Total CHO %	Fiber %	Ash %
Wheat						
Hard red spring	13	14	2.2	69.1	2.3	1.7
Hard red winter	12.5	12.3	1.8	71.7	2.3	1.7
Soft red winter	14	10.2	2	72.1	2.3	1.7
White	11.5	9.4	2	75.4	1.9	1.7
Durum	13	12.7	2.5	70.1	1.8	1.7
Flour, straight						
Hard wheat	12	11.8	1.2	74.5	0.4	0.46
Soft wheat	12	9.7	1	76.9	0.4	0.42
Flour, patent						
Bread	12	11.8	1.1	74.7	0.3	0.44
Germ	11	25.2	10	49.5	2.5	4.3

Source: Refs. 28–30.

Table 34 Breadmaking Functional Ingredients

Kind	Examples
Basic ingredient	
Wheat flour	Bread flour, whole wheat flour
Yeast	Compressed yeast, granular yeast, cream yeast, dried yeast, instant yeast, encapsulated yeast, frozen yeast, pizza yeast, deactivated yeast *Saccharomyces cervisiae*, *S. carlsburgensis*, *S. exisguus*
Salt	
Water	
Optional ingredients	Whole wheat flour, gluten, soya flour, wheat bran, other cereals or seeds, milk powder, fat, malt flour, egg, dried fruit, vitamins Sourdough bacteria: *Lactobacillus plantarum*, *L. brevis*, *L. fermentum*, *L. sanfrancisco* Other yeasts
Additives	
Emulsifier	Diacetylated tartaric acid esters of mono- and di-glycerides of fatty acids (DATA esters), Sodium stearyl-2-lactylate (SSL), distilled monoglyceride, lecithin
Flour treatment agents	Ascorbic acid, L-cysteine, potassium bromate, potassium iodate, azodicarbonamide
Preservatives	Acetic acid, potassium acetate, sodium diacetate, sorbic acid, potassium sorbate, calcium sorbate, propionic acid, sodium propionate, calcium propionate, potassium propionate
Processing aids	Alpha-amylase, hemicellulose, proteinase, novel enzyme systems (lipases, oxidases, peroxidases)

Source: Refs. 28–30.

Table 35 Basic Steps in Regular or Common Bread Making

Preparation of basic and optional ingredients
Preparation of yeast or sourdough for inoculation
Mixing of proper ingredients to make dough
Fermentation
Remixing of dough (optional)
Sheeting
Molding and panning
Proofing in a temperature- and relative humidity–controlled chamber
Decorative cutting of dough surface (optional)
Baking, steaming, frying, or boiling
Cooling
Packaging
Storage

Source: Refs. 28–30.

C. Retarded Dough

As indicated earlier, retarded dough is also available to some consumers. This type of dough is more available where refrigerators and freezers are more common. Dough is prepared in a way so that the fermentation is carefully controlled, and the dough is packed inside a container. Storage is also carefully controlled. When the package is opened, consumers can just follow the instructions to bake their own bread. The technology is proprietary to the manufacturers, but some guidelines are available (Table 38) (28–30).

D. Flat (Layered) Bread

Flat bread is a general term for bread products that do not rise as much as regular bread. They are common in Middle East countries and in countries or areas with less access to energy. In developed countries, flat breads are considered specialty breads. The making of the dough is similar to regular bread. But the dough is flattened and sometimes layered before baking directly inside the hearth or in an oven. Table 39 lists the general production scheme for flat breads (31,32).

E. Croissants and Danish Pastries

Croissants and Danish pastries can be considered products resulting from modification of the basic bread-making process. The dough preparation steps are similar but the ingredients are different. Table 40 compares the ingredients used in making croissants and Danish pastries. From this table, it is clear that even within each group, the formulation can vary considerably in the use of some ingredients, producing a wide variety of products available in the market (28–30).

F. Steamed Bread (Mantou)

Steamed bread is common in the Chinese community. Plain steamed bread is consumed as the major staple in the northern provinces of China. However, stuffed steamed breads are

Table 36 Various Breadmaking Processes

Straight dough baking process:
Weigh out all ingredients
Add all ingredients to mixing bowl
Mix to optimum development
First fermentation, 100 min., room temperature, or at 27°C for 1.5 hr
Punch
Second fermentation, 55 min, room temperature, or at 27°C for 1.5 hr
Divide
Intermediate proofing, 25 min, 30–35°C, 85% R.H.
Mold and pan
Final proofing, 55 min at 30–35°C, 85% R.H.
Bake at 191–232°C for 18–35 min. to ca. 100°C internal temperature
Sponge-and-dough baking process:
Weigh out all ingredients.
Mix part of flour, part of water, yeast and yeast food to a loose dough (not developed)
Ferment 3–5 hr at room temperature, or at 21°C for 12–16 hr
Add other ingredients and mix to optimum development
Fermentation (floor time), 40 min
Divide
Intermediate proofing, 20 min, 30–35°C, 85% R.H., or 27°C for 30 min
Mold and pan
Final proofing, 55 min, 30–35°C, 85% R.H.
Baking at 191–232°C for 18–35 min to ca. 100°C internal temperature
Continuous-baking process:
Weigh out all ingredients
Mix yeast, water, and maybe part of flour to form liquid sponge
Add remaining flour and other dry ingredients
Mix in dough incorporator
Ferment 2–4 hr, 27°C
Pump dough to development chamber
Dough development under pressure of 80 psi
Extrude within 1 min with 14.5°C and pan
Proof for 90 min
Bake at 191–232°C for 18–35 min to ca. 100°C internal temperature

Source: Refs. 28–30.

consumed as specialty items in various parts of China. Manufacture of steamed bread differs from that of regular bread mainly in the dough solidification process. Regular bread uses a baking process, whereas in steamed bread, steaming is used. Consequently, in steamed bread, there is no brown crust on the bread surface because the temperature used is not high enough to cause the browning reaction. Steamed bread is always consumed hot or held in a steamer as the bread is soft at this temperature. Sometime it is deep-fried before consumption. The bread hardens when it cools down, making it less palatable. Various procedures are available for production of steamed bread. Table 41 lists the basic steps in steamed bread processing in China (38).

Table 37 Sample Bread Recipes

White pan bread (bulk fermentation or straight dough process) Ingredients	Percent (on flour weight)
Flour	100.0
Yeast	1.0
Salt	2.0
Water	57.0
Optional dough improving ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

White pan bread (sponge and dough process) Sponge ingredient	Percent (of total flour)
Flour	25.0
Yeast	0.7
Salt	0.5
Water	14.0

Dough ingredients	Percent (of total flour)
Flour	75.0
Yeast	2.0
Salt	1.5
Water	44.0
Optional improving ingredients	
Fat	0.7
Soya flour	0.7
Malt flour	0.2

Source: Refs. 28–30.

Table 38 General Guidelines for Retarded Dough Production

Reduce yeast levels as storage times increase.
Keep yeast levels constant when using separate retarders and provers.
Reduce yeast levels as the dough radius increases.
Reduce yeast levels with higher storage temperatures.
The lower the yeast level used, the longer the proof time will be to a given dough piece volume.
Yeast levels should not be normally be less than 50% of the level used in scratch production.
For doughs stored below $-5^{\circ}C$, the yeast level may need to be increased.
Reduce the storage temperature to reduce expansion and weight loss from all dough pieces.
Lower the yeast levels to reduce expansion and weight losses at all storage temperatures.
Dough pieces of large radius are more susceptible to the effects of storage temperatures.
The lower freezing rate achieved in most retarder-provers combined with the poor thermal conductivity of dough can cause quality losses.
Prove dough pieces of large radius at a lower temperature than those of small radius.
Lower the yeast level in the dough to lengthen the final proof time and to help minimize temperature differentials.
Maintain a high relative humidity in proof to prevent skinning.

Source: Refs. 28–30.

Table 39 General Production Scheme for Flat Bread

Ingredient preparation
Mixing of ingredients (dough formation)
Fermentation
Dough cutting and rounding
Extrusion and sheeting (optional)
First proofing
Flattening and layering
Second proofing
Second pressing (optional)
Baking or steaming
Cooling
Packaging and distribution

Source: Refs. 31 and 32.

V. FERMENTED SOY PRODUCTS

A. Products and Ingredients

Soybeans have been available to the Chinese for centuries, and various fermented soy products were developed there and spread to neighboring countries. These countries further developed their own fermented soy products. Soy sauce, which originated in China, probably is the most famous and widely accepted fermented soy product. Credit also goes to the Kikkoman Company of Japan, which helps the spread of soy sauce worldwide through its marketing strategy. Fermented whole soybeans such as ordinary natto, salted soybeans (e.g., Japanese Hama-natto and Chinese Dou-chi), and tempeh (Indonesia), fermented soy pastes (e.g., Japanese miso and Chinese Dou-pan-chiang), and fermented tofus (e.g., sufu and stinky tofu or chao-tofu of Chinese origin) are more acceptable to certain ethnic groups. Consumers worldwide are gradually accepting these products through cultural exchange activities. The manufacturing of these products vary widely. Table 42 summarizes the ingredients needed for the manufacture of common fermented soy products (34).

Table 40 Formulations for Croissant and Danish Pastries

Flour (100%)	Croissants	Danish pastries
Flour	100	100
Salt	1.8–2.0	1.1–1.56
Water	52–55.4	43.6–52
Yeast (compressed)	4–5.5	6–7.6
Shortening	2–9.7	6.3–12.5
Sugar	2–10	9.2–25
Egg	0–24	5–25
Skimmed milk powder	3–6.5	4–6.25
Laminating margarine/butter	32–57	50–64

Source: Refs. 28–30.

Table 41 Basic Steps in Steamed Bread Processing

Selection of flour and ingredients such as milk powder and sugar (optional)
Mixing of dough
Fermentation:
Full fermentation—1–3 hr
Partial fermentation—0.5–1.5 hr
No-time fermentation—0 hr
Remixed fermentation dough—remixing of fully fermented dough with up to 40% of flour by weight
Neutralization with 40% sodium bicarbonate and remixing
Molding
Proofing at 40°C for 30–40 min (no-time dough)
Steaming for about 20 min
Steamed bread is maintained at least warm to preserve quality.

Source: Ref. 38.

B. Soy Sauce

There are many types of soy sauce, depending on the ratio of ingredients (wheat and soybeans), fermentation and extraction procedures, and flavoring ingredients (caramel and others) used. However, the procedures for their manufacture are similar. Basically, soy sauce is made by fermenting cooked soybeans in salt or brine under controlled condition to hydrolyze the soy proteins and starches into smaller flavoring components. The soy sauce is then extracted from the fermented soybeans for standardization and packaging. Table 43 lists a generalized scheme for the manufacture of soy sauce. More detailed information is presented in the chapter on soy sauce in this book and in references listed in this chapter (34–37,40).

C. Fermented Whole Soybeans

1. Ordinary (Itohiki) Natto

Ordinary natto is a typical Japanese fermented whole soybean product. Its sticky mucilageous substance on the surface of soybeans is its characteristic. It is produced by fermenting cooked soybeans with *Bacillus natto* for a brief period. It has a short shelf life. Table 44 lists the basic steps in the manufacture of ordinary natto. For detailed information on ordinary natto, please refer to the Chapter 30 in this book and the references in this chapter (34,36,41).

2. Hama-natto and Dou-chi

Hama-natto is fermented whole soybeans produced in the Hama-matsu area of Japan. Similar products are produced in Japan with different names prefixed with the production location. A very similar product in the Chinese culture is *tou-chi*, or *dou-chi*. It is produced by fermenting the cooked soybeans in salt, brine, or soy sauce, and then later drying them as individual beans. Hama-natto also includes the flavoring ginger. Table 45 lists the basic steps in the production of Hama-natto and dou-chi. For further information, readers should refer to Chapter 30 of this book and references in this chapter (34–36,41).

Table 42 Raw Ingredients for Fermented Soy Products

Ingredient	Soy sauce	Natto	Soy nuggets	Soy paste	Tempeh	Soy cheese	Stinky tofu
Major ingredients:							
Soy							
Soy bean	Yes	Yes	Yes	Optional	Yes	Yes	Yes
Soybean flour	Optional	No	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	No	Yes	No
Wheat	Optional	No	No	No	No	No	No
Rice flour	No	No	No	Optional	No	No	No
Major microorganism(s):							
Mold							
Aspergillus oryzae	Yes	No	Yes	Yes	No	Optional	No
Aspergillus sojae	No	No	No	Optional	No	No	No
Mucor hiemalis, *M. silivaticus*,	No	No	No	No	No	Yes	No
M. piaini	No	No	No	No	No	Yes	No
Actinomucor elegans, *A repens*,	No	No	No	No	No	Yes	No
A. taiwanensis	No	No	No	No	No	Yes	No
Rhizopus oligosporus,	No	No	No	No	Yes	No	No
R. chinensis var. *chungyuen*	No	No	No	No	No	Yes	No
Bacteria							
Bacillus natto	No	Yes	No	No	No	No	No
Klebsiella pneumoniae	No	No	No	No	Yes	No	No
Bacillus sp.	No	No	No	No	No	No	Yes
Streptococcus sp.	No	No	No	No	No	No	Yes
Enterococcus sp.	No	No	No	No	No	No	Yes
Lactobacillus sp.	No	No	No	No	No	No	Yes
Halophilic yeasts							
Saccharomyces rouxii	Yes	No	Yes	Yes	No	No	No
Torulopsis versatlis	Yes	No	Yes	Yes	No	No	No
Halophilic lactic bacteria							
Pediococcus halophilus	Yes	No	Yes	Yes	No	No	No
Bacillus subtilus	Yes	No	Yes	Yes	No	No	No
Additional flavor added	Optional	No	No	No	No	Optional	No
Preservative added	Optional	No	No	No	No	No	No

Source: Refs. 34–41, Chapter 31.

D. Fermented Soy Pastes

Both Chinese and Japanese have fermented soy pastes available in their cultures and they are made in a similar manner. However, the usage of these two products is quite different. The Japanese use fermented soy paste, miso, basically in making their miso soup, and to a lesser extent, for example, in the marinating/flavoring of fish. Miso soup is common in traditional Japanese meals. The Chinese use fermented soy paste, dou-pan-chiang, mainly as a condiment in food preparation. Dou-pan-chiang can also be made from wing beans, and this is beyond the scope of this chapter. Table 46 lists the basic steps in the manufacture of miso. For detail information on miso and dou-pan-chiang, readers should consult the references for this chapter and Chapter 30 on fermented whole soybeans and pastes in this book and other references in this chapter (34–37,39).

Table 43 Production Scheme for Soy Sauce

Selection and soaking of beans
Cooking of clean or defatted soybean, pressurized steam cooking at 1.8 kg/cm^2 for 5 min
Cooling of cooked bean to 40°C
Roasting and crushing of wheat
Mixing of prepared soybeans and wheat
Inoculation with *Aspergillus oryzae* or *sojae*
Incubation of mixture to make starter koji at 28–40°C
Addition of brine (23% salt water) to make moromi (mash)
Inoculation with halophilic yeasts and lactic acid bacteria (optional)
Brine fermentation at 15–28°C
Addition of saccharified rice koji (optional)
Aging of moromi (optional)
Separation of raw soy sauce by pressing or natural gravity
Refining
Addition of preservative and caramel (option)
Packaging and storage

Source: Refs. 34–36 37, 40.

E. Fermented Tofu

1. Sufu or Fermented Soy Cheese

Sufu or fermented soy cheese is made from fermenting tofu that is made by coagulating the soy protein in soy milk with calcium and/or magnesium sulfate. It is similar to feta cheese in its fermentation process. Both products are matured in brine in sealed containers. Some packed sufu contains flavoring ingredients. Table 47 lists the basic steps in the manufacture of sufu. For detailed information, readers should refer to the list of references in this chapter and Chapter 31 on fermented tofu in this handbook (34–36).

Table 44 Production Scheme for Itohiki (Ordinary) Natto

Cleaning whole soybean
Washing and soaking at 21–25°C for 10–30 hr
Pressurized steam cooking of soybean at 1–1.5 kg/cm^2 for 20–30 min
Draining and cooling of soybean at 80°C
Inoculation with *Bacillus natto*
Mixing and packaging in small packages
Incubation:
40–43°C for 12–20 hr, or
38°C for 20 hours plus 5°C for 24 hr
Final product
Refrigeration to prolong shelf life

Source: Refs. 34, 36, and 41.

Table 45 Production Scheme for Soy Nuggets (Hama-natto and Dou-chi)

Cleaning of whole soybean
Washing and soaking for 3–4 hr at 20°C
Steam cooking of soybean at ambient pressure for 5–6 hr, or at 0.81.0 kg/cm^2 for 30–40 min
Draining and cooling of soybean to 40°C
Addition of alum (optional for dou-chi)
Mixing with wheat flour (optional for Hama-natto)
Inoculation with *Aspergillus oryzae*

Procedure #1 (Hama-natto)
Incubation for 50 hr at 30–33°C
Soaking of inoculated soybean in flavoring solution for 8 months
Incubation under slight pressure in closed containers

Procedure #2 (Dou-chi)
Incubation at 35–40°C for 5 days
Washing
Incubation for 5–6 days at 35°C
Beans removed from liquid for drying
Mixing with ginger soaked in soy sauce (Hama-natto only)
Final product (soy nuggets)
Packaging
Refrigeration to prolong shelf life (optional)

Source: Refs. 34–36, 41.

Table 46 Production Scheme of Fermented Soybean Pastes (Miso)

Whole, clean soybean
Washing and soaking at 15°C for 8 hr
Cooking at 121°C for 45–50 min or equivalent
Cooling and mashing the soybeans
Preparation of soaked, cooked, and cooled rice (optional)
Preparation of parched barley (optional)
Inoculation of rice or barley with *Aspergillus oryzae* (tane-koji, optional)
Mixing of koji and rice or barley mixture
Addition and mixing of salt to koji and rice or barley mixture
Inoculation of halophilic yeasts and lactic acid bacteria (optional)
Packing of mixture (mashed soybean and koji) into fermenting vat with 20–21% salt brine
Fermentation at 25–30°C for 50–70 days
Blending and crashing of ripened miso
Addition of preservative and colorant (optional)
Pasteurization (optional)
Packaging and storage

Source: Ref. 34–37, 39–41, and Chapter 31.

Table 47 Production Scheme for Sufu (Chinese Soy Cheese)

Cleaning of whole soybean
Soaking
Grinding with water
Straining through cheesecloth to recover soymilk
Heating to boil and then cooling
Coagulation of soymilk with calcium and/or magnesium sulfate
Cooling to 50°C
Pressing to remove water (formation of tofu)
Sterilizing at 100°C for 10 min in hot-air oven
Inoculation with *Mucor*, *Actinomucor*, and/or *Rhizopus* sp.
Procedure #1
Incubation in dry form for 2–7 days depending on inocula
Incubation (fermentation in 25–30% salt brine) for 1 month or longer
Brined and aged in small containers with or without addition of alcohol or other flavoring ingredients
Procedure #2
Incubation at 35°C for 7 days until covered with yellow mold
Packed in closed container with 8% brine and 3% alcohol
Fermentation at room temperature for 6–12 months
Final product (sufu or Chinese soy cheese)

Source: Refs. 34–36, Chapter 31.

2. Stinky Tofu

Stinky tofu is a traditional Chinese food made by fermenting tofu in a "stinky brine" briefly. The tofu is hydrolyzed slightly during this brief fermentation and develops its characteristic flavoring compounds. When this raw stinky tofu is deep-fried, these compounds volatilize and produce the characteristic stinky odor, thus the name of "stinky tofu." It is usually consumed with chili and soy sauces. Stinky tofu is also steamed with condiments for consumption. Table 48 lists the basic steps in the manufacturing of stinky tofu. Readers should consult Chapter 31 in this handbook for further reading (34–36).

Table 48 Production Scheme for Stinky Tofu

Cleaning of whole soybean
Soaking
Grinding with water
Straining through cheesecloth to recover soymilk
Heating to boil and then cooling
Coagulation of soymilk with calcium and/or magnesium sulfate
Cooling to 50°C
Pressing to remove water (formation of tofu)
Pressing to remove additional water
Soaking in fermentation liquid for 4–20 hr at 5–30°C
Fresh stinky tofu ready for frying or steaming
Refrigeration to prolong shelf life

Source: Refs. 34–36, Chapter 31.

F. Tempeh

Tempeh is a traditional Indonesian food made by fermenting cooked soybeans wrapped in wilted banana leaves or plastic wraps. The mold *Rhizopus oligosporus* produces its mycelia and these mycelia penetrate into a block of soybeans. The mold mycelia also surround the block. This kind of fermentation is similar to molded cheese fermentation. Tempe has gradually been accepted by vegetarians in the West as a nutritious and healthy food. It is generally consumed as a deep-fried product. Table 49 lists the basic steps in the production of tempeh (34,36,38,41).

VI. FERMENTED VEGETABLES

A. Products and Ingredients

Fermented vegetables were produced in different cultures in the old days to preserve the harvested vegetables for them when they were not available or limited due to climatic condition. Some of these products grew from traditional cultural foods to foods widely accepted in other cultures. It is interesting that most of these processes are similar. Salt is used in the production of the product or the salt stock. Natural lactic acid fermentation is the major microbial activity in these processes, producing enough lactic acid to lower the pH. With the amount of salt added, these two ingredients create an environment that can inhibit the growth of other spoilage microorganisms. Available leafy vegetables, fruits (commonly used as vegetables), and roots are used as the raw materials. Starter cultures are used occasionally. Vinegar is used in some products. Chili pepper and other spices are used in many products. Preservatives may also be used to extend the shelf life after the package is opened. Table 50 compares the ingredients used in different fermented vegetable products (43,52).

B. Sauerkraut

The term sauerkraut literally means sour (sauer) cabbage (kraut). It is a traditional German fermented vegetable product that has spread to other cultures and is used in food preparations. The sequential growth of lactic acid bacteria in sauerkraut has long been recognized. Each lactic acid bacterium dominates the fermentation until its end product becomes inhibitory for its own development and creates another environment suitable for

Table 49 Production Scheme for Tempeh

Whole, clean soybeans
Rehydration in hot water at 93°C for 10 min
Dehulling
Soaking with or without lactic acid overnight
Boiling for 68 min
Draining and cooling to 38°C
Inoculation with *Rhizopus oligosporus* w/o *Klebsiella pneumoniae*
Incubation on trays at 35–38°C, 75–78 % R.H. for 18 hr
Dehydration
Wrapping

Source: Refs. 34, 36, 38, and 41.

Table 50 Raw Ingredients for Fermented Vegetables

Ingredient	Sauerkraut	Western pickles	Jalapeño peppers	Kimchi	Oriental vegetables
Vegetable					
Head cabbage	Yes	No	No	Optional	Optional
Chinese cabbage	No	No	No	Major	Optional
Mustard green	No	No	No	Optional	Optional
Turnip	No	No	No	Optional	Optional
Jalapeño pepper	No	No	Yes	Optional	Optional
Chili pepper	No	No	No	Yes	Optional
Pickle/cucumber	No	Yes	No	Optional	Optional
Salt	Yes	Yes	Yes	Yes	Yes
Starter culture (lactic acid bacteria)	Optional	Optional	Optional	No	No
Added vinegar	No	Yes	Yes	No	Optional
Added spices	No	Optional	Optional	Optional	Optional
Other added flavors	No	Yes	No	Optional	Optional
Preservative(s)	No	Optional	Optional	Optional	Optional

Source: Refs. 43–52.

another lactic acid bacterium to take over. The fermentation continues until most of the available fermentable sugars are exhausted. The production of sauerkraut is not risk-free, and sanitary precautions have to be taken to avoid spoilage. Table 51 presents the basic steps in sauerkraut processing (43,47,48,52).

C. Pickles

Western-style pickles are produced by salting the pickling cucumbers in vats into salt stocks for long-term storage, followed by desalting, and bottling in sugar and vinegar with or without spices. The fermentation is still lactic acid fermentation. However, it is more susceptible to spoilage because air may be trapped inside the slightly wax-coated cucumbers. In the salt curing of cucumbers, spoilage can occur and precautions should be taken to avoid its occurrence. Because of their high acidity and low pH, the products are gen-

Table 51 Basic Steps in Sauerkraut Processing

Selection and trimming of white head cabbage
Coring and shredding of head cabbage to 1/8 inch (31.25 mm) thick
Salting with 2.25 to 2.50% salt by weight with thorough mixing
Storage of salted cabbage in vats with plastic cover weighed with water to exclude air in the cabbage
Fermentation at 7 to 23°C for 2–3 months or longer to achieve an acidity of 2.0% (lactic)
Heating of kraut to 73.9°C before filling the cans or jars, followed by exhausting, sealing, and cooling
Storage and distribution

Source: Refs. 43, 47, 48, and 52.

erally mildly heat-treated to sterilize or pasteurize the product. Table 52 lists the basic steps in the production of Western-style pickles (43–47).

D. Kimchi

Kimchi is a Korean traditional fermented vegetable. Most kimchi is characterized by its hot taste due to the use of fairly high amount of chili pepper and its visibility in the product. However, some kimchi are made without chili pepper, but with garlic and ginger together with vegetables and other ingredients. It still has a hot taste from the garlic and ginger. Vegetables used in making kimchi vary with its formulations, with Chinese cabbage, cucumber, and large turnip being more common. Beside chili pepper, garlic and ginger can also be used to provide the hot sensation. Other ingredients may also be added to provide a typical flavor. The fermentation is still lactic acid fermentation. Traditionally, kimchi was made in every household in Korean rural areas to provide the vegetables for the winter when other fresh vegetables are not readily available. Nowadays, it is a big industry in Korea, with kimchi available all year round. In other parts of the world where Koreans live, kimchi resident, kimchi is available either as a household item or as a commercial product. Kimchi is usually not heat sterilized after packaging into jars. Pasteurization is optional. It is considered perishable and stored refrigerated. Table 53 lists the basic steps in the manufacture of kimchi (49,51).

E. Chinese Pickled Vegetables

China also manufactures a wide range of pickled vegetables. Various kinds of vegetables are used as raw materials. The fermentation can be either dry salting or brining process, depending on the product to be manufactured. However, the fermentation is still lactic fermentation. The major difference between Chinese-style pickled vegetable products and Western-style pickles is that desalting is usually not practiced in the manufacturing of Chinese-style pickled vegetables. The desalting process is left to the consumers, if needed. Also, some Chinese-style vegetables are made into intermediate-moisture products that have no western-style counterparts. Table 54 lists some of the basic steps in the manufacturing of selected Chinese pickled vegetables (50,51).

Table 52 Basic Steps in Fermented Pickles Processing

Sizing and cleaning of cucumber
Preparation of 5% (low salt) or 10% brine (salt stock)
Curing (fermenting) of cucumber in brine for 1–6 weeks to 0.7 to 1.0% acidity (lactic) and pH of 3.4 to 3.6 dependent on temperature, with salinity maintained at desirable level (15% for salt stock); addition of sugar, starter culture, and spices optional
Recovery of pickles from brine followed by rinsing or desalting (salt stock)
Grading
Packing of pickles into jars and filling with vinegar, sugar, spices, and alum depending on formulation
Pasteurization at 74°C for 15 min followed by refrigerated storage, or exhausting to 74°C at cold point followed by sealing and cooling, or vacuum pack followed by heating at 74°C (cold point) for 15 min and then cooling
Storage and distribution

Source: Refs. 43–47, and 52.

Table 53 Basic Steps in Kimchi Processing

Selection of vegetables (Chinese cabbage, radish, cucumber, or others)
Washing of vegetables
Cutting of vegetables, if necessary
Preparation of 8–15% brine
Immersion of vegetables in brine for 2–7 hr to achieve 2–4% salt in vegetable
Rinsing and draining briefly
Addition of seasoning
Fermentation at 0°C to room temperature for about 3 days
Packaging (can also be conducted before fermentation)
Storage at 3–4°C

Source: Refs. 49 and 51.

VII. APPLICATION OF BIOTECHNOLOGY IN THE MANUFACTURING OF FERMENTED FOODS

With the advances in biotechnology, microorganisms with special characteristics for the manufacturing of fermented foods have become available. The most significant example is the approval by the FDA of Chy-Max (chymosin produced by genetic manipulation) used in the production of cheese. Its availability greatly reduces the reliance of chymosin from young calves, with economic savings. Other products with similar or other properties are also available in the market. Genetically modified lactic acid bacteria and yeasts used in the fermented food production are also available nowadays to reduce production cost. Gradual acceptance by consumers is the key to the further development and success of

Table 54 Basic Steps in Fermented Chinese Vegetables

Selection and cleaning of vegetables
Cutting of vegetables (optional)
Procedure #1
Wilting of vegetables for 1–2 days to remove moisture
Dry salting of vegetables in layers with weights on top (5–7.5% salt)
Fermentation for 3–10 days
Washing
Drying or pressing of fermented vegetables (optional)
Addition of spices and flavoring compounds
Packaging
Sterilization (optional)
Procedure #2
Wilting of cut vegetables
Hot water rinsing of fermentation container
Filling the container with cut vegetables
Addition of 2–3% brine and other flavoring compounds (optional)
Ferment at 20–25°C for 2–3 days
Ready for direct consumption or packaging and cool storage

Source: Refs. 50 and 51.

biotechnology (1–3,5,7,10,53–55). Readers should refer to other chapters in this handbook and other references available for further information.

VIII. PROCESS MECHANIZATION IN THE MANUFACTURE OF FERMENTED FOODS

Fermented foods produced by traditional methods are labor intensive and rely a great deal on the experience of the manufacturers. The main drawback is product inconsistency. Many cheeses, yogurts, breads, sausages, and soy sauce are now made by highly mechanized processes to standardize the products in most developed countries (57–65). This not only provides product consistency but also reduces production costs. Consumers benefit from these developments. However, some consumers, even in developed countries, still prefer the traditional products, even at increased cost, because of their unique product characteristics. There are also fermented products that are still being made by traditional or semi-mechanized processes because mechanization processes have not been developed for these products.

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5
Quality and Flavor of Fermented Products

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I. INTRODUCTION

Various fermented products originated from spontaneous fermentation of a raw material, such as milk, meat, or vegetables. Initially, the main focus was on preservation of the raw material. Raw milk, for example, will be susceptible to microbial spoilage at room temperature; the milk will rapidly acidify, due to the activity of lactic acid bacteria. Apart from preservation, fermented products also develop distinctive tastes and smells that are widely appreciated. Over time, in the process of fermentation many variations developed, partly purposely and partly spontaneously and in relation to the technology used. Their nature depends very much on the type of raw material used, on its pretreatment, on the temperature (climate), and on the conditions of fermentation. This practice is still common in various parts of the world: part of a spontaneously fermented product is transferred deliberately into a new batch of raw material in order to maintain or even strengthen it. This principle is the basis for controlled preservation, sustainable and safe, many times resulting in an attractive flavor, although the process is not controlled.

Many fermented products contain lactic acid bacteria, but other bacteria, yeasts, and molds may be involved as well. In order to be able to produce products with a more consistent quality, starter cultures for the preparation of fermented food have been developed. These cultures are composed of selected microorganisms, propagated as defined mixtures of pure cultures or as undefined mixtures of different types of bacteria. The use of starters, on the one hand, has been tremendously positive with respect to the quality of the product, but, on the other hand, it has decreased the diversity of fermented products. Because the food industry is keen to explore new possibilities for enhancing the diversity of its product range, there is a new interest nowadays in searching for potential starter organisms from the pool that existed at the time of spontaneous fermentations. This chapter gives an overview of various fermented products and the main organisms used with their roles in production. It focuses in particular on dairy fermentations, because these fermentations are among the best-studied cases, and information obtained from these studies can also be applied in other fermentations.

II. VARIOUS FERMENTED FOOD PRODUCTS AND STARTER ORGANISMS USED

The preparation of fermented foods is a practice with a very long history. Already thousands of years ago, for instance—as reported in the bible—wine and cheese were being made. In those times, hardly anything was known about the underlying mechanisms; however, many interesting products evolved. All of them were the result of spontaneous fermentations, essentially resulting in products with an extended shelf life and (often) flavorful characteristics. The extension of shelf life was obviously a major reason for fermentation practices. Later on, after the discovery the causal agents for the fermentation processes, these microorganisms were isolated and used in order to be able to produce products with a consistent quality. This led to the practice of using industrial starter cultures and gave the manufacturer more control over the technological, safety, and consistent quality aspects of their food products.

Many fermented products exist, and for each of them cultures with specific characteristics are involved. For instance, in the fermentation of meat, combinations of various bacterial species are used. In almost all cases, lactic acid bacteria are present in these starter cultures, with *Lactobacillus sakei, Lb. curvatus, Lb. plantarum, Pediococcus pentasaceus*, and *P. acidilactici* as most important species. Also, *Micrococcus varians*, *Staphylococcus carnosus*, and *S. xylosus* species are used. These microorganisms are able to grow under low pH (4.5–5.0) conditions, low water activities (A_w 0.9–0.95), anaerobic conditions, and in the presence of salt (2–3%). Not only do these organisms cause a decline in pH, but they also contribute to the typical flavor of fermented meat products.

A number of fermented meat products also exhibit a fermentation on the surface, resulting from the growth of yeasts (*Debaromyces hansenii, Candida famata*, and *C. utilis*) and molds (*Penicillium chrysogenum* and *P. nalgiovense*). The surface flora usually adds to the flavor formation through specific lipolytic and proteolytic activities.

For the production of bread and sourdoughs, yeast (*Saccharomyces cerevisiae*) and lactic acid bacteria (e.g., *Lactobacillus sanfransiscensis*, *Lb. amylovorum*) are employed. The required gas formation in these products largely is due to the sugar fermentation of the yeast cells, although also some heterofermentative lactobacilli can add to this. The fermentation of sourdough is more complex because it requires the interaction of various microorganisms. The various flavor components formed in these products, such as aldehydes, alcohols, and pyrols, are partly the result of biochemical conversions by the starter cultures but also result from the baking process itself (1,2).

The fermentation of vegetables, such as olives, cabbage, and pickles, belongs to the least developed practices. The production methods are still rather simple: basically, lactic acid bacteria such as *Lb. plantarum, Leuconostoc* spp, *Pediococcus*, and *Lactococcus* are used. The acid formation results in extended shelf life, and also flavor formation by the microorganisms adds to the overall quality of these products. For the fermentation of soy, which is a very common and traditional process in east Asia, various mold species are used, such as *Rhizopus oligosporus* and *R. oryzae*. These fermentations are often solid-state fermentations, for which the raw material is inoculated with microorganisms that grow at the surface of the raw material, making it essentially an aerobic fermentation process. For some of the products, there is an extensive proteolysis, leading to products like soy sauce, whereas in other fermentations (e.g., tempeh) only limited proteolysis occurs (3).

Wine and beer are fermented with various yeast strains (*S. cerevisiae*). In some wines, also *Leuconostoc oenos* is used. In these cases, the main pathway related to preservation is the formation of alcohol from sugar of the grain or the grapes, respectively. Again, as in

other fermentation processes, many other reactions occur that contribute to the final sensory quality of the products and that are in fact even more important for the final quality. In the preparation of malt for beer brewing, microorganisms are also employed in order to lower the pH and to reduce the outgrowth of spoilage organisms (e.g., certain molds) (4,5).

III. CULTURES USED FOR THE MANUFACTURE OF DAIRY PRODUCTS

All kind of lactic acid bacteria as well as other microorganisms are used for the manufacture of various dairy products. Because dairy fermentations are among the best-studied fermentations, with several new developments, we focus on starter cultures in these fermentations and their characteristics. This section will give examples of the main species used, various characteristics required for the quality of the final products, and flavor formation in particular. Many of the described characteristics are also of importance in other fermented products.

A. Lactic Acid Bacteria

When raw milk is left at room temperature for some time, a microflora will develop in which lactic acid bacteria generally dominate. These bacteria acidify the milk and, as a consequence, inhibit the growth of other bacteria. Concomitantly, they give the milk a pleasant flavor. This spontaneous fermentation has been the basis of several fermented dairy products in history. The discovery of the role of lactic acid bacteria in milk fermentation paved the way for their isolation, characterization, and exploitation. This started more than a century ago and has resulted in the development of starter cultures for the manufacture of fermented dairy products. Both industrial and small-scale manufacture now almost always rely on industrially prepared starters (6).

The lactic acid bacteria used in dairy fermentations can roughly be divided into two groups on the basis of their growth optimum. Mesophilic lactic acid bacteria have an optimum growth temperature between 20° and 30°C; thermophilic strains, between 30° and 45°C. Traditional fermented products from subtropical countries harbor mainly thermophilic lactic acid bacteria, whereas products with mesophilic bacteria originate mainly from western and northern European countries. Currently, most dairy industries use starter cultures for rapid acidification, because a spontaneous fermentation acidifies the milk too slowly. This method of working has reduced the number of strains present and is responsible for certain uniformity in dairy products. On the other hand, it has made production very reliable and efficient. The microorganisms present in natural niches still form a very interesting potential for product diversification, due to their large biodiversity (see below).

Industrial (dairy) starter cultures can be divided into two groups: undefined and defined starters. The undefined starters are a mixture of an unknown number of lactic acid bacteria types, which are originally derived from an artisanal production practice and further selected for the production of good-quality dairy products. Mesophilic undefined starter cultures are commonly used for the manufacture of Cheddar, Gouda, and other cheese types. Their use is based on their consistent performance, especially their well-recognized phage resistance (7,8).

Defined-strain starters are blends of two of more strains; in the case of Cheddar cheese, they are frequently used nowadays instead of the former undefined mixed-strain starters. The risk of phage attacks is greater here than with the use of undefined mixed-

strain cultures, so cultures with different phage sensitivity profiles are used in rotation (8–10). Another well-known example of a defined-strain starter is the mixture of thermophilic lactic acid bacteria in the manufacture of yogurt.

B. Lactococci

It is interesting to consider that the industrial starters for the majority of cheeses are based on a single mesophilic species, namely *Lactococcus lactis*. Strains of this species are employed for the manufacture of various cheese types and, although they exhibit different characteristics, they have several biochemical attributes in common (11,12). The most important properties are their ability to produce acid in milk and to form flavor components.

For the manufacture of Gouda cheese, the undefined mixed-strain starters are composed of acid-forming lactococci, *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, possibly in combination with the citrate-utilizing strains *L. lactis* subsp. *lactis* biovar *diacetylactis*, and *Leuconostoc* spp. (Fig. 1). These starters are thus composed of complex mixtures of strains, forming a bacterial population that is equipped with the properties suitable for the production of the desired cheese. Because their composition would change depending on the conditions of cultivation (13), their subculturing is minimized and the cultures are preserved by freezing or lyophilization.

Also, the manufacture of Cheddar cheese and several other cheese types is entirely dependent on the fermentative activity of lactococci, sometimes in the form of defined-strain starters, consisting of *L. lactis* subsp. *lactis* varieties in case of Cheddar cheese, but frequently still in the form of undefined mixed-strain starters.

Mesophilic lactococci are generally considered to be associated with the milk environment (14) but they can also be isolated from other sources (15). Lactococci isolated from artisanal manufacture of fermented dairy products without the application of industrially prepared starter cultures and from nondairy environments are generally referred to as

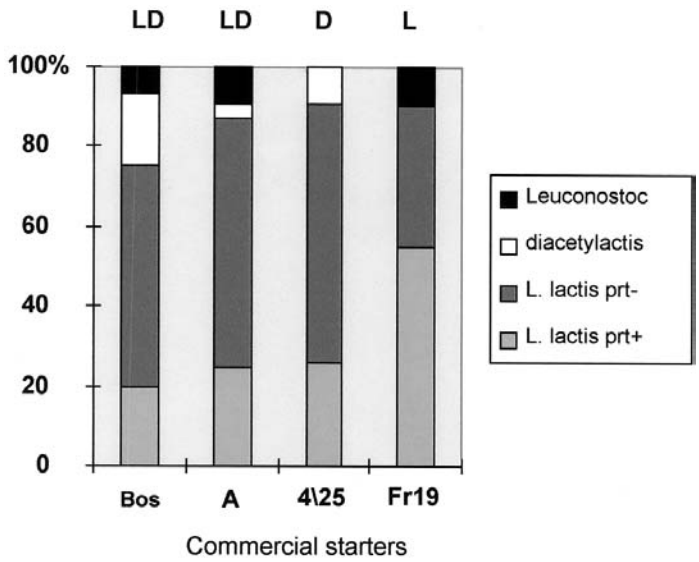

Figure 1 Composition of some commercial mixed-strain undefined starter cultures used for cheese making.

"wild" lactococci. In an international project funded by the European Community, many wild strains of lactic acid bacteria were isolated and partially characterized (16). Initial studies showed that this pool of lactic acid bacteria contained many *L. lactis* strains, which differ in a number of phenotypic properties from the strains commonly present in industrial starters. Among those characteristics is the ability to form flavor components (17–20). This aspect, interesting for diversification in the manufacture of fermented dairy products, will be discussed below.

C. Mesophilic Lactobacilli

Although mesophilic lactobacilli are undoubtedly inhabitants of raw milk and the dairy environment, upon acidification of raw milk they are frequently overgrown by strong acidifiers of the genus *Lactococcus*. However, they do gain access to the cheesemaking process, because they are often found as secondary flora during the ripening of different cheese varieties. This is especially true for raw-milk cheese, but mesophilic lactobacilli are also common in cheese manufactured with modern technologies, using pasteurization of the milk, defined-strain starters, and hygienic processing. The starter is responsible for the acidification during the first stages of cheese manufacture and may reach up to 10^9 colony-forming units (cfu) per gram of cheese. During ripening, however, the number of starter cfu generally decreases rather quickly to lower than 10^7 g^{-1}, whereas subsequently the non-starter adventitious lactobacilli grow out and may reach numbers higher than those of the starter culture (21).

The secondary flora in Cheddar cheese has been examined most extensively; it consists mostly of mesophilic lactobacilli and sometimes also pediococci. These bacteria are collectively referred to as nonstarter lactic acid bacteria (NSLAB). Isolates from this group belong to the species *Lactobacillus paracasei*, *Lb. plantarum*, *Lb. rhamnosus*, and *Lb. curvatus*. The composition of the NSLAB in the cheese varies with the day of manufacture and with the age of the cheese (16,22). Adventitious NSLAB have also been reported for Emmental, Comté, and other types of cheeses (23–25).

The NSLAB have the unique ability to grow under the highly selective conditions prevailing in a ripening cheese. Lactose is largely depleted in the first hours of cheese manufacture by the fermentation of the starter bacteria. The pH is between 4.9 and 5.3, the temperature below 13°C, the moisture content less than 50%, the salt concentration in moisture is 4–6%, and oxygen is barely available. All in all, ripening cheese seems a hostile environment for microorganisms. Yet the adventitious lactobacilli manage to grow, obviously with a low rate, but the generally long ripening period allows them enough time to reach considerably high levels of cfu per gram of cheese. They apparently consume compounds other than lactose, such as lactate, citrate, glycerol, amino sugars, amino acids, and perhaps even on material released from lysed starter bacteria. Although the NSLAB, like other lactobacilli, exhibit fastidious nutritional requirements, they clearly find ample opportunities for growth in a ripening cheese. They possess a wide range of hydrolytic enzymes and are able to effect proteolysis and lipolysis (22,26).

Because NSLAB dominate the microflora of many long-ripened cheeses, they are believed to contribute to the maturation of cheese. The numbers of NSLAB are reported to be higher in Cheddar cheeses made from raw milk than in those from pasteurized milk (27). Differences in flavor between these cheeses, with a more intense flavor in raw milk cheeses, suggest that the indigenous NSLAB play an important role in flavor development. Indeed, they have been shown to contribute to the formation of small peptides and amino acids, which are the precursors of the flavor components (28).

The observation that the presence of NSLAB in cheese on the one hand leads to a desirable flavor and on the other hand may induce possible defects or spoilage (29–31) makes it a delicate choice for the cheese maker to use a certain lactobacillus as adjunct starter. This strain should be selected with care because only a limited number of the NSLAB present in cheese combine all the required properties with the concomitant lack of imperfections. McSweeney et al. (32) were successful in improving the flavor of Cheddar cheese by using strains isolated from raw milk cheese. This improvement was believed to be due to increased formation of amino acids. Cheese made from milk inoculated with strains of *Lb. plantarum* or *Lb. casei* subsp. *pseudoplantarum* received the best gradings (33).

D. Thermophilic Lactic Acid Bacteria

The thermophilic lactic acid bacteria are best known as starters for fermented milks. Several varieties of fermented milks originate from countries in Asia Minor and the Balkans (e.g., Bulgarian yogurts). These yogurt products have emerged from spontaneous acidification of raw milk by indigenous organisms and have now been developed into microbiologically well controlled industrial processes. The two most frequently used starter bacteria are classified as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, generally shortened as *Lb. bulgaricus* and *S. thermophilus*, respectively.

Yogurt is usually made by inoculating an equal mixture of *S. thermophilus* and *Lb. bulgaricus* in milk and incubating it at 37–45°C. *S. thermophilus* grows optimally at these temperatures and hydrolyzes lactose via a β-galactosidase. *S. thermophilus* is nutritionally fastidious and requires a complex mixture of amino acids for growth. Because it is proteolytic negative, the coupling with the more proteolytic *Lb. bulgaricus* stimulates its growth in milk. The production of formic acid and carbon dioxide from lactose by *S. thermophilus*, on the other hand, stimulates the growth of *Lb. bulgaricus* (Fig. 2) (34).

Thermophilic lactic acid bacteria also play an essential role in the manufacture of some cheese types. The starters of Swiss-type and Italian cheeses consist mainly of *S. thermophilus, Lb. helveticus*, and *Lb. bulgaricus* (35,36). Also, in the ripening of Greek hard cheese types made from ewes' and goats' milk, thermophilic lactic acid bacteria play a dominant role (37). The specific high cooking temperature used in the manufacture creates their niche in these cheese types. They convert lactose to lactic acid as in all dairy fermentations. This acid plays its usual role as preservative and, for Swiss-type cheeses, is the substrate for the subsequent propionic acid fermentation, leading to large eye formation in the cheeses.

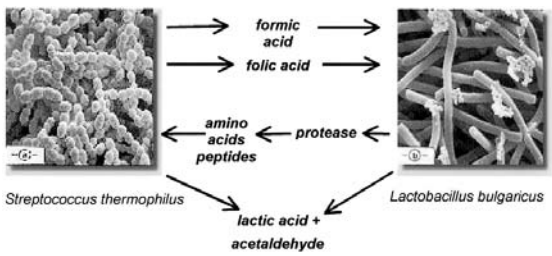

Figure 2 Proto-cooperation between *Streptrococcus thermophilus* and *Lactobacillus bulgaricus* in a yogurt culture. This symbiosis results in an increased lactic acid and acetaldehyde (yogurt flavor) production.

Because thermophilic lactic acid bacteria were found to be very active, they are employed as adjunct cultures in the manufacture of various cheeses. In some cases they are specifically selected to address certain flavor aspects of cheese (e.g., bitterness). Smit et al. (38–40) reported on thermophilic lactic acid bacteria with high debittering activities, which were found to be very useful in debittering of cheeses made with a mesophilic starter culture that causes bitterness (Fig. 3). Various thermophilic adjunct cultures are also employed to enhance the formation of desired flavor notes in a given cheese.

E. Yeasts

Although yeasts play a minor role in dairy fermentations, several fermented milk products with a natural yeast–containing microflora exist. A distinctive feature of these products is that, in addition to the lactic acid fermentation induced by lactic acid bacteria, a slight alcoholic fermentation due to yeasts takes place (41,42). The best-known examples of fermentation of milk by a combination of yeasts and lactic acid bacteria are kefir and kumiss, both originating from countries of Eastern Europe and Asia. The predominant species of yeast in kefir and kumiss are *Kluyveromyces marxianus, Candida kefir*, *Saccharomyces cerevisiae*, and *Saccharomyces delbrueckii*. The heterogeneity of the species found can be explained by the different techniques of kefir grain cultivation. Yeasts play an important role in promoting symbiosis among the microorganisms present, CO_2 formation, and development of the characteristic taste and aroma (43).

Yeasts are also involved in the ripening of surface-ripened cheeses. Their primary role is to lower the acidity of the cheese surface, which allows the development of a secondary microbial flora (44,45). Positive interactions between yeasts and starter organisms are well documented for semi-soft cheeses, such as Limburger and Tilsit (46). The yeasts metabolize lactate, which causes an increase of the pH, and may secrete factors that promote the growth of *Brevibacterium linens*, which is essential for the ripening of these cheeses (45). Yeasts are also believed to promote the development of *Penicillium roqueforti* in blue cheeses. They may contribute to the open structure of these cheeses by formation of gas (47). On the surface of Camembert, the dominating yeast flora inhibit the growth of

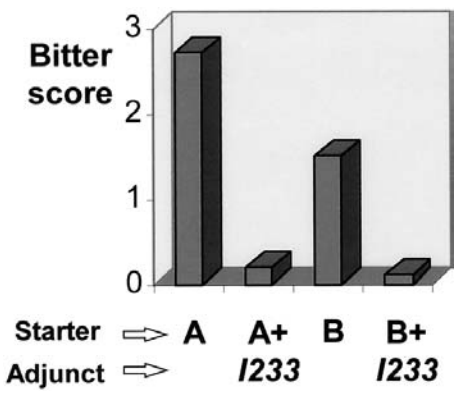

Figure 3 Effect of addition of the thermophilic adjunct culture I233 on the flavor development of a Gouda cheese after 3 months of ripening. The application of this adjunct culture results in a strong decrease in the bitter score during sensory evaluations. Starters A and B are known for the production of bitter-tasting peptides during cheese ripening.

the spoilage molds. The most prominent yeasts in cheese are *Debaromyces hansenii, Kluyveromyces marxianus, Kluyveromyces lactis, Yarrowia lipolytica, Saccharomyces cerevisiae*, and *Zygosaccharomyces rouxii* (48,49).

F. Molds

Molds are mainly used in the manufacture of semi-soft cheese varieties together with the lactic acidifiers. Their major role is to enhance the flavor and aroma and to modify the body and the structure of cheese. On the basis of their color and growth characteristics, they can be divided into white and blue molds. The former type grows on the outside of the cheese (e.g., Camembert and Brie) and is known as *Penicillium camemberti*. The blue mold is named *Penicillium roqueforti* and grows inside the cheese. Examples of blue cheeses are Roquefort, Blue Stilton, Danish Blue, and Gorgonzola (50). Several varieties of these two *Penicillium* species have been described in the past, but they should all be considered as biotypes. The characteristic feature of mold-ripened cheese is the extensive proteolysis and lipolysis. These biochemical activities ultimately result in the formation of precursors for the typical volatile flavor components of the cheeses. Methyl ketones have a key role in the typical flavor. There is a positive correlation between the free fatty acid level and the amount of methyl ketones formed, and cheeses with limited lipolysis score lower in flavor (51,52). Other typical compounds besides methyl ketones are secondary alcohols, esters, aldehydes, and lactones. Various compounds arising from the proteolysis and amino acid conversion complete the aroma of these cheeses. Clearly, the flavor of mold-ripened cheese is a delicate balance of several compounds produced by a succession of microorganisms, which each performs its particular activity. Not only the choice of the *Penicillium* strain is important for the successful production of the soft surface mold cheeses; selection of the concomitant starters is also crucial.

Geotrichum candidum is used as one of the starters for the manufacture of the Finnish fermented milk viili and Camembert cheese. The starter of viili consists of mesophilic lactococci and *Leuconostoc* strains with *G. candidum*. Viili is made from nonhomogenized milk, and the fungus forms a moldy layer on the cream. Its growth is generally limited due to the restricted amount of oxygen present in the container with the fermenting mixture (53). *G. candidum* is one of the contributors to the flora that plays a key role in the ripening of Camembert cheese. It starts to grow on the surface of the rind of Camembert, Pont l'Evêque, and Livarot cheeses at the beginning of the ripening process and contributes to typical cheese flavors (54,55). Most notably, *G. candidum* is known for its potential to form sulfur-containing flavor components, which contribute to the typical flavor characteristics of Camembert cheeses (56–58).

G. Other Bacteria

Finally, some other groups of bacteria, which have not been covered above, are nevertheless worth mentioning. The propionic acid bacteria constitute the essential secondary flora in Swiss-type cheese. After homofermentative lactic acid fermentation, they convert lactate into propionate, acetate, and carbon dioxide. The latter is responsible for the characteristic eye formation in these cheeses. Interest in the role of propionic acid bacteria in flavor formation has recently been renewed (59). Also for this group of bacteria, the raw milk environment appears to be a versatile and interesting source of strain variation (60).

A second group of bacteria, which play a major role in the maturation of surface-ripened cheese, comprises the brevibacteria and other coryneform bacteria. They are ob-

viously present in the smear of these cheeses and are strongly proteolytic, which results in high levels of sulfur-containing volatiles (45,61). They also show some lipolytic and esterolytic activity, and produce distinct red-orange pigments (62). The variation in proteolytic activity, antimicrobial activity, and pigment biosynthesis in these bacteria may offer opportunities for the selection of appropriate variants for specific applications in the manufacture of smear-ripened cheeses or even other types of cheeses (63).

IV. STARTER FUNCTIONALITIES

As listed above, various microorganisms are used as a starter culture for the production of fermented products (e.g., dairy products). Apart from acidification and thus preservation, it is found that these organisms often possess certain characteristics that offer specific benefits for the consumer. These benefits are now leading in the development of new and improved starter cultures. A number of specific characteristics will be discussed below. Special attention is paid to the flavor-forming abilities of starter cultures because flavor perception is the most prominent aspect for the consumer when choosing a food product.

A. Bacteriocins

Different lactic acid bacteria are able to produce bacteriocins, proteinaceous substances with bactericidal activity against microorganisms closely related to the producer strain (64–67). Lactic acid bacteria are generally regarded as safe microorganisms and so are their bacteriocins. Thus, these bacteriocins can potentially be used to control the growth of spoilage and pathogenic organisms in food (68). Bacteriocin-producing lactococcal strains have been used successfully in starter cultures for cheese-making in order to improve the safety and quality of the cheese (69–71). For instance, the use of nisin-producing starter cultures was found to effectively reduce the spoilage by *Clostridium tyrobutyricum* strains (Fig. 4).

Recently it was found that the production of bacteriocins and bacteriocin-like compounds is a property that is very common among strains isolated from natural sources (15,16,72,73). This phenomenon can possibly be explained by the fact that the ability to produce antimicrobial compounds offers these wild strains the power to withstand the competition of other microorganisms and thus to survive in their hostile natural environment. The bacteriocin-producing wild lactococcal strains may be useful as starters in cheese making, not only because of their antimicrobial activity but also because of their potential to synthesize interesting flavor compounds (see below). These strains should then be combined with other strains that are bacteriocin-resistant.

B. Vitamin Production

Lactic acid bacteria are able to produce certain vitamins, for in yogurt a higher concentration of folic acid is found than in milk (74). *S. thermophilus* is known to produce folic acid during growth in milk (75,76). The amount of folic acid found in cows' milk ranges from 20 to 60 μg L^{-1}, whereas its concentration in yogurt may be increased depending on the strains used for the fermentation and on the storage conditions to values above 200 μg L^{-1}. This level appears also to be dependent on the strain of *Lb. bulgaricus* used, because the latter organism has been shown to use and to degrade folic acid during its growth (77). It is therefore of utmost importance to select the optimal combination of *S. thermophilus* and *Lb. bulgaricus* strains leading to organoleptically acceptable yogurt

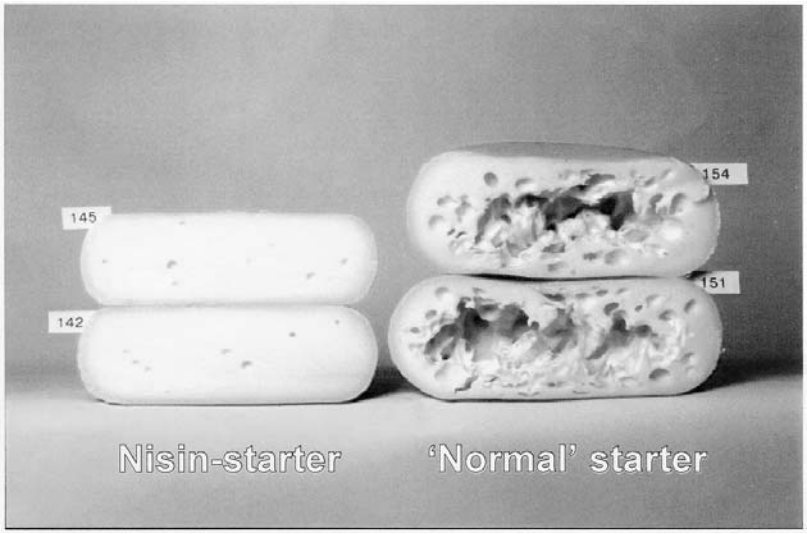

Figure 4 Effect of a nisin-producing starter culture on the reduction of growth of *Clostridium tyrobutyricum* after 12 weeks of ripening. In the absence of nisin ("normal" starter), this bacterium causes excessive gas formation, leading to large holes in the cheese and a strong off-flavor.

with concomitantly an increased folic acid concentration. The pools of available strains will undoubtedly harbor such strains.

C. Exopolysaccharides

By producing exopolysaccharides, both *S. thermophilus* and *Lb. bulgaricus* contribute to the viscosity and the smooth texture of fermented products like yogurt. They stabilize the gel and decrease its tendency to synerise (78). These bacteria produce rather low amounts of exopolysaccharides with a wide variety of chemical structures. Glucose, galactose and rhamnose are its main monomers, and the composition might be affected by the fermentation conditions (79,80). The chemical structure of the polymer apparently has an effect on the rheological properties, because the use of different starters results in differences in its microscopic structure and viscosity (81). The underlying mechanism of this effect, however, is not well understood. The emerging knowledge on the genetics of exopolysaccharide biosynthesis by *L. lactis* and *S. thermophilus* strains (Fig. 5) (82,83) will offer opportunities to modify the structure of the polymer and to regulate the amount synthesised.

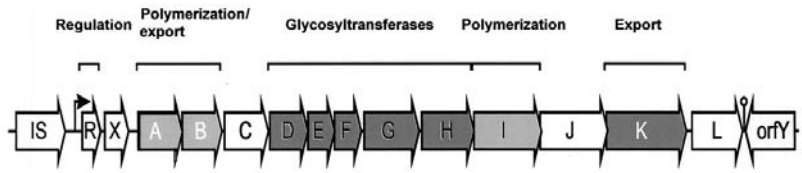

Figure 5 Genetic map of the genes involved in EPS production in *L. lactis*. (From Ref. 83.)

D. Eye Formation

Lactic acid bacteria are able to ferment citrate, which is present in many raw materials used for food fermentations such as fruit, vegetables, and milk. Citrate can be fermented by a limited amount of mesophilic lactic acid bacteria [(84,85) for a review]. Its degradation results in acetate, diacetyl, acetoin, 2,3-butanediol, and CO_2. Besides the formation of the flavor component diacetyl, which is a crucial flavor attribute in buttermilk, ripened cream butter, sour cream, and quarg, the formation of CO_2 is responsible for eye formation in cheese.

In the production of Swiss-type of cheeses, such as Gruyere, Emmental, and Maasdammer, propionibacteria are used to achieve the characteristic sweet and nutty flavor notes as well as the large eyes. Various metabolic pathways have been described for the utilization of lactate as energy source and aspartate as electron acceptor (86). The eye-formation in Swiss-type cheese can be controlled by a combination of aspartase-weak propionibacteria and facultative heterofermentative lactobacilli (87).

E. Probiotic Features of Starter Cultures

Probiotics are live microbial food supplements that benefit the health of consumers by maintaining or improving their intestinal microbial balance (88). Species of *Lactobacillus* and *Streptococcus* have traditionally been used in fluid fermented dairy products to promote human health (89). These probiotic starters may influence the microbial ecology of the host, lactose intolerance, incidence of diarrhea, mucosal immune response, levels of cholesterol, and cancer. Besides the traditional carrier of probiotics, which is yogurt, the market share of probiotic drinks is expanding rapidly. The most common probiotic strains are lactobacilli. A number of them are now being successfully commercialized, such as *Lactobacillus rhamnosus* GG (90). *Lb. casei* Shirota (91) and *L. acidophilus* LA-1 (92). The scientific basis of these strains in relation to their proposed positive effects on the human health is quite extensive; however, most of the evidence is obtained from diseased human populations. Evidence for probiotic claims in healthy populations still has a weak basis.

V. FORMATION OF FLAVOR COMPOUNDS DURING FERMENTATIONS

Flavor development in fermented products results from a series of (bio)chemical processes in which the starter cultures provide many of the enzymatic activities. Particularly, the enzymatic degradation of proteins leads to the formation of key-flavor components, which contribute to the sensory perception of the products (93–95). In the following section, the flavor-forming enzymes of lactic acid bacteria will be discussed, with dairy products as the main example. On the other hand, it is important to note that very similar activities can be found in other fermented products, such as meat and sourdoughs.

The formation of flavors in fermented dairy products is a complex and, in the case of cheese ripening, rather slow process involving various chemical and biochemical conversions of milk components. Three main pathways can be identified: the conversions of lactose (glycolysis), fat (lipolysis), and caseins (proteolysis) (Fig. 6). The enzymes involved in these pathways are predominantly derived from the starter cultures used in these fermentations.

In the case of the lactose fermentation, the main conversion obviously leads to the formation of lactate by lactic acid bacteria, but a fraction of the intermediate pyruvate can

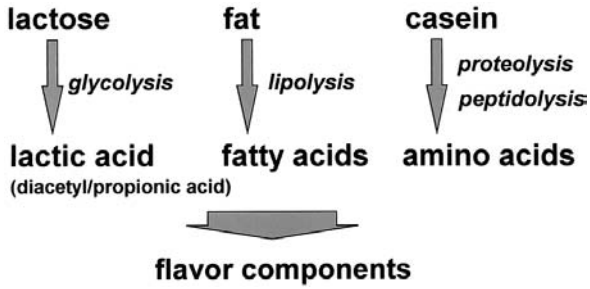

Figure 6 General conversions of the milk constituents during the ripening of cheese. Three main pathways can be distinguished. The pathway from casein is the most important one, leading to typical cheese flavors.

alternatively be converted to various flavor compounds such as diacetyl, acetoin, acetaldehyde, or acetic acid, some of which contribute to typical yogurt flavors.

Lipolysis results in the formation of free fatty acids, which can be precursors of flavor compounds such as methylketones, alcohols, and lactones. Lactic acid bacteria contribute relatively little to lipolysis, but additional cultures (e.g., molds in the case of surface-ripened cheeses) (96) often have high activities in fat conversion. Flavors derived from the conversion of fat are particularly important in soft cheeses like Camembert and Roquefort.

The conversion of caseins is undoubtedly the most important biochemical pathway for flavor formation in hard-type and semi-hard-type cheeses (97). Degradation of caseins by the activities of rennet enzymes, and the cell-envelope proteinase and peptidases from lactic acid bacteria, yields small peptides and free amino acids. A good balance between proteolysis and peptidolysis prevents the formation of bitterness in cheese (38,39). Although it is known that peptides can taste bitter and that amino acids can taste sweet, bitter, or broth-like (98), the direct contribution of peptides and amino acids to flavor is probably limited to a basic taste (93). For specific flavor development, further conversion of amino acids is required to various alcohols, aldehydes, acids, esters, and sulfur compounds. The current knowledge on these pathways is shown in Fig. 7.

A. Proteolysis Leads to Peptides and Free Amino Acids

Because the concentrations of free amino acids and peptides are very low in milk, the starter cultures depend for growth in milk heavily on their proteolytic systems. The degradation of milk proteins (caseins) leads to peptides and free amino acids, which can subsequently be taken up by the cells (99,100). Proteolysis is initiated by a single cell-wall-bound extracellular proteinase (Prt), which can be either chromosomally or plasmid-encoded. Most dairy lactic acid bacteria strains contain such an extracellular proteinase, but several do not and these are mainly dependent on other strains in the starter culture for the production of peptides and amino acids. Such dependency of strains is rather common in starter cultures and indicates the relevance of knowledge on the population dynamics between strains in order to be able to develop stable starter cultures.

Peptide and amino acid transport systems have been studied extensively in lactococci, but far less is known for other lactic acid bacteria such as mesophilic and thermophilic lactobacilli [see for a review Kunji et al. (99)]. Peptide and amino acid uptake occurs via oligopeptide transport systems, and di-/tripeptide transporters as well as various amino acid

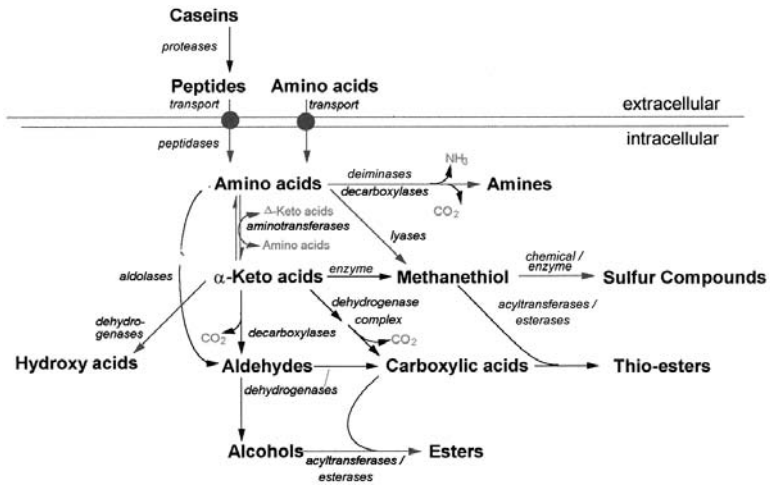

Figure 7 Pathways from amino acids to flavor compounds. (From Ref. 97)

transport systems (101). Following uptake, the peptides are degraded intracellularly by a variety of peptidases (99,100). These peptidases of lactic acid bacteria can be divided into endopeptidases, aminopeptidases, di-/tripeptidases, and proline-specific peptidases. The specialized peptidases in lactic acid bacteria for hydrolysis of Pro-containing peptides have been postulated to be important for the degradation of casein-derived peptides, because these are known to have a high proline content.

The balance between the formation of peptides and their subsequent degradation into free amino acids is very important. Because accumulation of peptides might lead to a bitter off-flavor in cheese (38,39,102,103). Various bitter-tasting peptides have been identified and especially these peptides should be degraded rapidly in order to prevent off-flavors (102–104). Specific cultures have been selected with high bitter-tasting peptide degrading abilities (38,39) and such cultures are nowadays frequently used in the preparation of various types of cheese (Fig. 3).

The ability of cultures to degrade bitter-tasting peptides was not only found to be dependent on the strain used, but also on the growth conditions. For example, it was found that pH-controlled growth conditions resulted in a higher debittering activity (40). The mechanism behind these differences was found to be strongly correlated with the sensitivity of the cells to lysis. Meijer et al. (105) showed that the introduction of a transposon in *L. lactis* SK110 not only increased the stability of the cells, but also the bitterness in cheese made with this culture. These results indicate that the cell membrane can be a barrier between the enzymes, located intracellularly, and the peptide substrates, present in the cheese matrix. Apparently, there is not enough active transport anymore with the starter cultures once they are present in the cheese matrix for a certain ripening time.

B. Amino Acid Converting Enzymes (AACEs)

Amino acids are the precursors of various volatile cheese flavor compounds that have been identified in cheese (93,94). They can be converted in many different ways by enzymes such

as deaminases, decarboxylases, transaminases (aminotransferases), and lyases (Fig. 7). Transamination of amino acids results in the formation of α-keto acids that can be converted into aldehydes by decarboxylation and, subsequently, into alcohols or carboxylic acids by dehydrogenation. Many of these components are odor-active and contribute to the overall flavor of the dairy product. Moreover, other reactions may occur—for example, by hydrogenase activity toward α-keto acids resulting in the formation of hydroxyacids, which do hardly contribute to the flavor.

Using biochemical and genetic tools, various flavor-forming routes from amino acids and enzymes involved have recently been identified, mostly in *L. lactis* [see (95,97,106) for a review].

Aromatic amino acids, branched-chain amino acids, and methionine are the most relevant substrates for cheese flavor development. Conversion of aromatic amino acids can result in formation of undesirable flavors, so-called off-flavors, such as *p*-cresol, phenylethanol, phenylacetaldehyde, indole, and skatole, which contribute to putrid, fecal, or unclean flavors in cheese (100). Many of these reactions can also occur under cheese conditions and are highly dependent on the strain used (107). This implies that by target selection of starter bacteria the formation of undesirable flavors can be avoided. A similar strain dependency is also found for enzyme activities that result in the formation of desired flavor compounds, indicating that a strong potential for starter improvement exists (see below).

Conversion of tryptophan or phenylalanine can also lead to benzaldehyde formation. This compound is found in various hard-type and soft-type cheeses and contributes positively to the overall flavor (94,96). In *Lb. plantarum* as well as in other lactic acid bacteria, the formation of benzaldehyde out of phenylalanine is initiated by an aminotransferase reaction followed by a chemical conversion of the intermediate phenylpyruvic acid into benzaldehyde (108,109). The latter reaction requires the presence of manganese, for which an efficient uptake system was found (110). This chemical conversion occurs at a high pH and in the presence of oxygen, so it is not very likely a main conversion pathway in cheese.

Branched-chain amino acids are precursors of various aroma compounds such as isobutyrate, isovalerate, 3-methylbutanal, 2-methylbutanal, and 2-methylpropanal. These compounds are found in various cheese types. Several enzymes that are able to convert these amino acids have been detected in *L. lactis* [see (95,97,106) for a review]. The aromatic aminotransferases can convert aromatic amino acids, but also leucine and methionine; the branched-chain aminotransferases can convert the branched-chain amino acids leucine, isoleucine, and valine, but also methionine, cysteine, and phenylalanine.

Volatile sulfur compounds derived from methionine, such as methanethiol, dimethylsulfide, and dimethyltrisulfide, are regarded as essential components in many cheese varieties (111). In fact, a Gouda cheese-like flavor can be generated by incubation of methionine with cell extracts of *L. lactis* (93). Conversion of methionine can occur via a aminotransferase-initiated pathway by branched-chain or aromatic aminotransferases, or via an α,γ-elimination of methionine by the lyase activities of cystathionine β-lyase (CBL), cystathionine γ-lyase (CGL), or methionine γ-lyase (MGL) (97,107,112–121).

It was found that the amount of α-keto acids determines the rate of the first step in the conversion of amino acids. Overproduction of the transaminases alone did not lead to a strong increase in amino acid conversion without a simultaneous addition of keto acids as co-substrate (122). The introduction of a glutamate dehydrogenase gene from *Peptostreptococcus* in *L. lactis* resulted in a similar effect (123). However, whether this activity also results in a strong increase in the desired flavor components remains to be determined.

Ayad et al. (20) found that also the presence of enzymes required for subsequent conversions might be of crucial importance (Fig. 8).

Although cystathionine lyases are active under cheese-ripening conditions (112), their activity towards methionine could not be detected using ^{13}C nuclear magnetic resonance (107). With this technique, only the aminotransferase-initiated pathway was observed suggesting that this pathway is most prominent in methionine catabolism to produce methanethiol. On the other hand, strains that overproduce cystathionine β-lyase, where found to be able to degrade methionine, indicating the potential of this enzyme in the production of sulfury flavors (120,121). The specificity of CBL (110) is a particular advantage in this respect, since one might expect that only sulfury flavor components would increase in strains with high activity. In case of overproduction of other less-specific enzymes such as transaminases, more pathways will be influenced at the same time.

Biosynthesis and degradation of some amino acids are intricately coupled pathways. For instance, the above mentioned cystathionine β-lyase can convert methionine to various volatile flavor compounds, but in bacteria its physiological function is the conversion of cystathionine to homocysteine, which is the penultimate step of methionine biosynthesis (97). This means that many of the AACEs are in fact involved in the biosynthesis of amino acids. It is well known that biosynthesis of amino acids is highly regulated, and therefore the growth conditions of the starter cultures might affect their flavor-forming capacities. For instance, in *L. lactis* the expression of the gene coding for cystathionine β-lyase is strongly influenced by the amounts of methionine and cysteine in the culture medium (120,121). High concentrations of these amino acids completely abolish transcription and result in *L. lactis* cells almost deficient of cystathionine β-lyase activity.

Similarly, it is found that the branched-chain aminotransferase is also regulated at the transcriptional level in *L. lactis* (124). The physiological role of branched-chain aminotransferases in bacterial metabolism is to catalyze the last step in the biosynthesis of branched-chain or aromatic amino acids. Several enzymes can thus been considered as being involved in both biosynthesis and degradation of amino acids, and α-keto acids are intermediates in both directions. These examples illustrate that the choice of culture conditions can strongly influence the flavor-forming capacities of starter cultures like *L. lactis*.

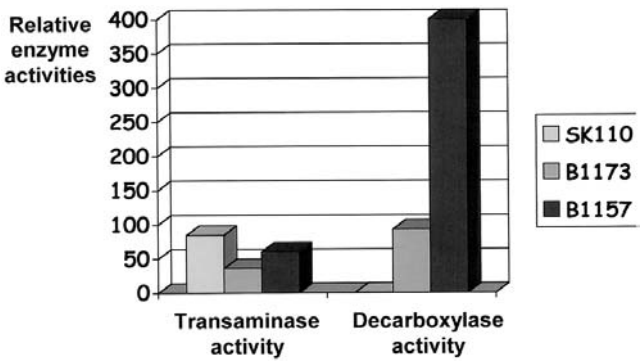

Figure 8 Variation in enzyme activities between industrial (SK110) and wild *L. lactis* (B1173 and B1157) strains in the two-step conversion of leucine towards 3-methyl aldehyde. The largest difference was found in step 2: the decarboxylation of the ketoacid towards the aldehyde.

C. Natural Biodiversity

It is already mentioned that various lactic acid bacterial strains differ in amino acid converting abilities and that these activities are in fact linked to the ability to synthesize amino acids (18,19) focused on the flavor-forming and the amino acid forming abilities of *Lactococcus* strains isolated from various natural sources, the so-called wild lactococci (Fig. 8). These strains originated from dairy and nondairy environments, and they were found to have unique properties when compared to commercially available starter strains. For instance, many of these strains do not degrade caseins, produce antimicrobial compounds, and/or have low acidifying activity. When the amino acids–forming capacities of these strains were determined using the single omission technique (125), it was found that these strains had a much larger potential to synthesize amino acids as compared to industrial strains. Lactococci used in dairy fermentations are known for their limited capacity for biosynthesis of amino acids, which explains their complex nutritional requirements; they require several amino acids for growth (18,126–128). For instance, most dairy *Lactococcus* strains need glutamate, valine, methionine, histidine, serine, leucine and isoleucine. Industrial *L. lactis* subsp. *cremoris* strains require even more different amino acids for growth (18). Wild *L. lactis* subsp. *cremoris* strains generally require two to three amino acids while some *L. lactis* subsp. *lactis* strains only need one amino acid. The absence of some amino acid biosynthetic pathways in dairy lactococci might be a consequence of their adaptation to dairy products because in milk, the amino acids are readily available from the proteolytic degradation of caseins. Wild strains are not naturally associated with a rich environment such as milk, which makes them more dependent on their own biosynthesis of amino acids compared to industrial strains. The large natural biodiversity that is found within lactic acid bacteria species offers good possibilities for flavor diversification. It can obviously also be applied in various other fermented food products.

VI. CONCLUSIONS AND FUTURE PERSPECTIVES

Fermented products bring the consumer a sense of appreciation, which is by and large due to the interaction of the right starters cultures, technology, and care in manufacturing. The starter cultures play a specific role in this respect and for that reason deserve specific attention. Not only are the starter cultures responsible for preservation, due to lowering of the pH or increase of ethanol, but they also form various important compounds, most notably flavor components, which contribute directly to the perception of the consumers.

This knowledge makes it necessary to put special emphasis on the research in the area of starter cultures and their functionalities. Especially in view of the natural biodiversity that still exists in food-grade microorganisms, it is important to preserve this pool of microbial strains for future applications. The product innovator is challenged to use strains from them in his trials to create the flavor or other attributes the consumer demands.

The variation in microbes and their intrinsic biochemical potential is huge in size. This will make it necessary to have a better understanding of the enzymatic pathways in these starter cultures, in order to be able to select strains with specific desired characteristics. For instance, the rapidly growing knowledge of pathways prevailing in these bacteria that are active in flavor formation offers new insights in the required properties of a given starter culture. This should lead to the design of probes to screen collection of microbes (pools of genes) very effectively for the presence of certain desired traits. Moreover, apart from the best-studied examples of starter cultures (e.g., the lactococci) other

microorganisms are receiving more attention recently. This will even enlarge the potential possibilities in this field. An interesting time is ahead of us, not only from a scientific point of view, but also from the perspective of manufacturers of fermented products.

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6

Semisolid Cultured Dairy Products: Principles and Applications

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I. SEMISOLID CULTURED DAIRY PRODUCTS

A. Introduction

Fermented semisolid dairy foods have a long history of consumption worldwide. This category of dairy foods, which includes yogurt, sour cream, fromage frais and many variations, continues to show increased sales because of the healthful reputation (of these products) and their convenience. The $2.9 billion yogurt industry saw a 10.5% increase in sales from 1999 to 2000 (1). Within the yogurt category, new product entries are continually appearing in marketplaces that promote probiotic-enhanced benefits. Innovative packaging concepts that target convenience and fun also drive the market surge.

Classification of fermented semisolid dairy products is difficult because of the wide variety of products that can be manufactured. Traditional dairy products such as cheese varieties have been classified based on moisture content (2). Although widely used, this system suffers a serious drawback in that dairy products with different characteristics and manufacturing protocol are grouped together. In other attempts, products are classified based on similarities that exist, such as starting ingredients, the type of fermentation, and rheology (3). However, it is important to define rheology, which is the mathematics of a set of behaviors rather than a set of materials (4). Rheology of a product is related to the flow of fluids and the deformation of matter. Texture is defined as the tendency to resist flow and is often used interchangeably with rheology. Typically, texture relates to solid foods and viscosity relates to fluid products. Many foods can exhibit characteristics of both solid and liquid (5). Fluid milk and cream are considered liquids, and hard cheeses are classified as solids. Semisolid fermented dairy foods show an intermediate behavior, with properties of both solids and liquids, and can be defined as viscoelastic (6). Because viscoelasticity characteristics are very dependent on the protein network structure formed by casein micelles (6), a great deal of research has focused on processing parameters that optimize a network structure that favors good rheological properties and desired firmness without syneresis.

In this chapter, yogurt, fromage frais, and sour cream are considered semisolid fermented dairy products based on the consensus in the literature. Typical manufacturing protocol for these products require raw milk selection, milk pretreatments, ingredient blending and standardization, heat processing, starter addition, and other technological proce-

dures to produce satisfactory products with desired body, texture, flavor, and organoleptic and rheological properties. Keeping these aspects in mind, principles and applications of fermented milk manufacture are discussed under two sections: section I discusses selection of raw milk, milk pretreatments, ingredient addition, heat processing, role of lactic starter system, gel formation during fermentation, and proactive phage control program. Section II is devoted to probiotics in semisolid dairy foods, covering health issues, probiotic cultures, and product development and production of probiotic dairy foods.

B. Selection of Raw Milk

As the old saying goes, there is no substitute for good-quality raw milk. This is also true for milk destined for fermented milk manufacture. Quality criteria for raw milk should be based on compositional quality of milk, microbial count, somatic cell count, freedom from inhibitory substances, and reception temperature (7). In the United States, grade A milk is required for fermented milk manufacture. Such milk has standard plate count of less than 100,000 cfu/mL and storage temperature not exceeding 7°C (within 2 hr of milking). Somatic cells are an indicator of udder health, and individual states have set limits for acceptable somatic cell count (8). Generally, a somatic cell count above 750,000/mL is not acceptable. High somatic cell count is indicative of mastitis conditions; milk may also be abnormal in composition. Leukocytes in milk may cause inhibition of lactic starters by phagocytosis, and abnormal composition can influence other technological parameters affecting quality of resultant fermented milks.

Good-quality raw milk is free from all kinds of inhibitory substances, including residual antibiotics and traces of detergents, sanitizers, or insecticides. Treatment of mastitis in cows involves application of various antibiotics, and milk from treated cows may occasionally contain residual antibiotics if the milk is not withheld for the recommended period. The resulting low-level contamination may be sufficient to inhibit starter microorganisms (9); for example, penicillin at a concentration of 0.01 IU/mL of milk will inhibit thermophilic starters. Apart from the possibility of partial or complete inhibition of starter bacteria due to antibiotic action, antibiotic testing is also mandatory for consumer safety reasons due to potential allergic reaction by sensitive individuals (10). High levels of residual detergents and sanitizers can inhibit starter bacteria. Under Good Sanitation Practices, the amount of residual sanitizers that might enter into milk from milk contact surfaces is not sufficient to cause culture inhibition except when sanitizer solution is not completely drained from tanks or trucks (11). Quarternary ammonium compounds (QAC) present a potential problem due to their residual activity, and lactic acid bacteria are sensitive to low concentrations of QAC. For example, *Lactococcus* strains are inhibited at 10 μg/mL level of QAC (12) and thermophilic cultures are inhibited at 0.5 to 2 μg/mL of QAC (13).

C. Milk Pretreatments

Commonly employed pretreatments in the manufacture of fermented milk are filtration, clarification, and homogenization.

1. Filtration

Filtration is carried out by pumping milk through specially woven cloth. This results in the removal of extraneous matter. Filtration of the recombined dairy powders is also helpful in

the removal of undissolved or scorched particles from the base mix. This may be further helpful in minimizing wear and tear to the homogenizer orifice and minimizing the deposition of milk solids on the heat exchanger plates.

2. Clarification

Clarification refers to the process of centrifugal separation carried out in a specially designed separator, called a clarifier. This process removes extraneous matter such as straw, hay, and large clumps of bacteria and thereby improves the esthetic quality of fermented milks. A specially designed centrifuge can be used to carry out bactofugation. This process can reduce bacterial load by about 80–90%. It is especially effective for removal of spores and leukocytes from milk (~98%).

3. Homogenization

Homogenization is a process of attaining homogeneous emulsions. This is achieved by passing the milk under high pressure through a small orifice; the shearing effect reduces the average diameter of the milk fat globules to less than 2 μm. As a result, the globules are less inclined to coalesce to large units and do not rise to form a cream line.

Homogenization is an important processing step for improving consistency and viscosity of yogurt due to incorporation of the finely divided fat globules within the coagulum structure. Further, coagulum stability also improves due to adsorption of casein micelle onto fat globules. The increased number of small fat globules tend to enhance the ability of the milk to reflect light, and as a result the fermented milk appears whiter. Use of unhomogenized milk leads to fat accumulation on top of the packaged product, which is disliked by most customers except in products where a cream line is specifically required (e.g., crusty yogurt in Greece).

Milk processing for fermented milk manufacture typically employs homogenization pressure in the range of 10–20 MPa at 50–70°C temperature. Homogenization preceding heat processing is preferable to avoid risk of contamination after heat processing.

D. Ingredient Addition

Optional ingredients can be added to the base mix to improve viscosity and to minimize syneresis in fermented milk products. Additionally, with the development of low-fat dairy products, stabilizers are often added to improve mouthfeel and to reduce calories (14).

1. Stabilizers

Stabilizers serve two basic functions when added to semisolid fermented dairy foods. Good stabilizer systems will have the ability to bind water, react with milk proteins to increase their level of hydration, and stabilize protein molecules into a network that prevents the movement of water. Gelatin and hydrocolloids such as guar gum, pectin, carageenan, and pregelatinized starch are the most common stabilizers (15). A stabilizer is added as a single component or as a blend. Blends of stabilizers have the benefit of overcoming a limiting property of a single stabilizer. It is important to balance the desired level of stabilizer with undesirable flavor changes the stabilizer may impart. The stabilizer concentration will vary widely depending on the type of system chosen. Processing conditions may need to be considered when choosing the stabilizer system.

2. Sweeteners

Products are typically sweetened by sucrose, honey, high fructose corn syrup (HFCS), or aspartame. Some manufacturers use apple and pear concentrates when a healthier image is desired (15).

3. Fruits, Colorants, and Flavorings

Addition of fruit, colorants, and flavorings are also common in fermented semisolid dairy products and are typically added after fermentation. Typically, heat-treated purees that range from 30 to 50° Brix are used. Because fruit is commonly added after the dairy product is heat-treated, the microbiological quality is critical to the safety and shelf life of the product. In set yogurts, a layer of gelled or pureed fruit is placed into the container prior to addition of cultured milk (14).

4. Other Ingredients

Today, other less common ingredients are making their way into fermented dairy products. Addition of vitamins and minerals has become a method for increasing nutritional value. Adding calcium to the fermented milk base has been difficult due to solubility and bioavailability issues. Recent patents for methods of increasing calcium in fermented dairy products focus on technology that overcomes the low solubility of calcium salts in milk (16).

5. Prebiotics

Prebiotics has been defined as "non-digestible food that beneficially affects the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon" (17). Prebiotics, such as oligosaccharides, are therefore used to maximize the effectiveness of probiotic microorganisms. Based on their chemical structure, some oligosaccharides are resistant to digestive enzymes and are bioavailable to *Bifidobacterium* in the large intestine as a carbon and energy source (18). Research suggests that fructo-oligosaccharides, lactose derivatives, galacto-oligosaccharides, and soybean oligosaccharides have commercial significance as prebiotic ingredients (19). Addition of the prebiotic fructo-oligosaccharide or galacto-oligosaccharide in combination with *Bifidobacterium* in yogurt has been shown to increase the intestinal probiotic level by 2 log cycles. The current trend in yogurts marketed in Australia, Europe, and Japan is for them to contain a prebiotic component (18).

E. Heat Processing

Pasteurization of milk is a legal requirement in the United States for manufacture of fresh cultured products (*CFR* Title 21, part 133:1995). Heat treatments typically employed for selected fermented milk products are depicted in Table 1.

The most important impact of heat processing is microbiological. Due to heat treatment, most vegetative bacteria, yeast, molds, and pathogens are destroyed ($\geq$5 log cycle reduction is required). This has a strong impact on safety and quality of the resultant product. Surviving spores are unlikely to cause problems, because *Bacillus* and *Clostridium* endospores do not germinate at the pH of most fermented milks (i.e., pH < 4.5) (20). Heat-stable enzymes are reported to not pose a significant problem in semisolid fermented milk products (21), with the exception of products like sour cream that may be critical for lipolytic or proteolytic spoilage. With regard to starter culture, reduced competition between

Table 1 Typical Heat Treatments Employed for Fermented Milk Preparations

Product	Typical heat treatment(s)	Reference
Yogurt	90–95°C for 2–5 min 80–85°C for 30 min	20
Sour cream	74°C for 30 min 85°C for 25 sec	21
	80°C for 30 min 90°C for 5 min	7
Fromage frais	93°C for 120 sec	22

surviving flora and starter organisms is beneficial for the growth of the latter. Due to the high heat processing employed for milk destined for fermented milk preparation, the germicidal activity of milk is destroyed along with its natural inhibitors, making conditions more suitable for starter growth. Severe heat treatment denatures whey proteins in milk, and α-lactalbumim and β-lactaglobulin become attached to casein micelles, especially k-casein. This leads to increased viscosity and enhanced water-binding capacity of coagulum (22).

F. Role of Lactic Starter System

In the limited context of fermented milks discussed in this chapter, starter culture systems can be defined as safe, pure, actively growing, selected lactic acid bacteria that bring about desirable changes to produce a satisfactory fermented product.

Typical starter microflora, inoculation rates, incubation temperature, and incubation periods employed in fermented milk manufacture are summarized in Table 2. Typical starter flora for yogurt include *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB). In the United States, the National Yogurt Association recommends the

Table 2 Typical Starter Microflora, Inoculation Rates, Incubation Temperature, and Incubation Period for Selected Fermented Milk Products

Product	Starter microflora	Inoculation rate	Incubation temperature	Incubation period
Yogurt	*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*	2–3%	40–45°C 30°C	2.5–4 hr 18 hr
Sour cream	*Lactococcus lactis* subsp. *lactis* and or *Lactococcus lactis* subsp. *cremoris* *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis Leuconostoc mesenteroides* subsp. *cremoris*	1–2%	18–21°C	14–20 hr
Fromage frais	*Lactococcus lactis* subsp. *lactis* and or *Lactococcus lactis* subsp. *cremoris* *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis Leuconostoc mesenteroides* subsp. *cremoris*	0.5–3%	27°C	14–16 hr

level of at least 10 million live yogurt bacteria per gram at the time of consumption if manufacturers wish to display the "Live and Active Cultures" symbol on yogurt packages (23).

The relationship between ST and LB is best described as that of protocooperation and antibiosis (24) as the former becomes stimulated by free amino acids and peptides produced by LB (25). In turn, LB is stimulated by formic acid, pyruvic acid, and carbon dioxide produced by ST at low oxygen concentration (26). As a result, acid production is significantly enhanced in mixed cultures compared to ST and LB grown individually, and yogurt gel tends to set faster. The unique relationship between the two starter organisms also makes yogurt flavor unique compared to other dairy products. The unique flavoring compounds in yogurt include acetaldehyde (optimum flavor and aroma at 23 to 41 ppm), acetone and butanone (contributed from milk), diacetyl, and acetoin (27). Presence of diacetyl (0.5 ppm) contributes to delicate and full flavor and aroma. Acetaldehyde production is mainly attributed to LB. However, in association with ST, acetaldehyde production is considerably enhanced as compared to LB alone (28).

Typical starter flora of sour cream or cultured cream includes *Lactococcus lactis* subsp. *lactis* (LL) and/or *Lactococcus lactis* subsp. *cremoris* (LC), *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (LD), *Leuconostoc mesenteroides* subsp. *cremoris* (LeuC). Main sensory attributes of cultured cream are sour taste (lactic acid), buttery aroma (due to diacetyl), and nutty flavor. Starter flora in sour cream exhibit positive (mutually beneficial) association. LD and LC can ferment citrate under aerobic conditions to produce diacetyl. Acetaldehyde in cultured cream is not desirable because it imparts a green flavor. In such cases, inclusion of LeuC in the starter system is advantageous because it scavenges acetaldehyde produced by lactococci. Conversely, *Leuconostoc* organism that lack proteolytic activity and do not grow well in milk grow better in association with lactococci.

During sour cream manufacture, when the citrate level is depleted, diacetyl is reduced to acetoin. Increasing oxygen content in milk may enhance production of diacetyl; however, addition of citrate in milk (0.05–0.1%) is a preferable option. Production of cultured cream requires proper selection of bacteria as well as manufacturing conditions to induce the right balance of acid and flavor because flavor producers are fastidious in their temperature requirement. If the temperature of incubation is maintained much above 22°C, insufficient diacetyl will be produced (29).

Purity and activity checks are very important for optimum performance of the starter system. Purity can be checked by microscopic examination of culture suspension or catalase test to detect contamination. Starter flora are typically gram-positive, catalase-negative, nonsporulating rods or cocci. Activity test or vitality test can be conducted by monitoring rate of acid development (30). For active cheese culture, acidity development is expected to be at least 0.4% lactic acid after 3.5 hr incubation at 37.8°C and 3% inoculation. For cultured cream and cheese cultures, activity test can be run at 30°C instead of 37.8°C. For checking aroma-producing starter bacteria in a mixed culture, Voges-Proskauer or creatine test can be used. Creatine can be used to confirm presence of aroma-producing cultures. It is also useful for detecting gas-producing contaminants in a non–gas-forming culture (31).

G. Gel Formation During Fermentation

During the manufacture of semisolid dairy foods, an irreversible gel is formed due to the destabilization of the casein micelle. The gel can be classified into one of the following groups based on whether it is (a) enzymatic, (b) acid-induced, or (c) salt/heat-induced (14). The gel or coagulum formation in fermented semisolid dairy foods is primarily of the acid

type similar to that of acid-set cheeses. In acid-set cheeses, however, syneresis is desirable, whereas in yogurt or other semisolid fermented foods it is not desirable. In studies investigating the acidification of skimmed milk with glucono-δ-lactone at 30°C, the following changes were observed in the casein micelles:

At pH 6.6–5.9, casein micelles were homogeneously distributed with no appearance of change.
At pH 5.5–5.2, appearance of micellar disintegration and at pH 5.2 or less, aggregation of casein micelles was observed.
At pH 5.2–4.8, casein aggregates contracted and micelles were much larger than the native form.
At pH < 4.5, protein network formed with rearrangement and aggregation of casein consisting of micellular chains and clusters with trapped water and milk constituents in the network (32).

H. Proactive Phage Control Program

A current trend in the fermented milk industry is consolidation of smaller plants into larger plants and increased production volume. Starter suppliers are now offering ranges of options such as direct vat set cultures or frozen concentrated cultures. As a result, the problem of bacteriophage and other microbiological risk factors have been reduced considerably. Nevertheless, preparation of bulk culture cannot be entirely eliminated due to certain practical considerations, and in such situations bacteriophage contamination remains a distinct possibility, especially during in-house handling.

In the light of this, a proactive phage-control program is necessary. Such a program can be very helpful in maintaining consistent starter performance and thereby assuring consistent quality of fermented milks.

Many of the strategies employed for bacteriophage control are summarized below.

Good whey handling and processing practices. Whey is a principal site where phage multiplication and buildup occurs.
Adequate heat treatment of bulk culture milk (e.g., 95°C for 30 min) to inactivate phages in milk.
Use of phage-resistant starter cultures or multiple strain starter or mixed strain starter used in rotation. Where feasible, direct vat inoculation with deep-frozen or lyophilized concentrated bulk cultures.
Use of phage-inhibitory media or phage-resistant media for bulk culture propagation.
Use of exopolysaccharide-producing strains that are more resistant to phages (33).
Use of mechanically protected (e.g., Lewis system, Jones system, Alfa Laval System) system to maintain asepsis and to avoid contamination of bulk culture.
Good sanitation practices to ensure freedom from phage buildup on vat surfaces and other potential milk contact surfaces. Chlorine is a better sanitizer than iodine or acid sanitizers for phage control.

II. PROBIOTICS IN SEMISOLID DAIRY FOODS

A. Introduction

In the early 1900s, Nobel prize–winning scientist Élie Metchnikoff was the first to propose that acid-producing organisms in fermented dairy foods offered a prolongation of life by

preventing fouling in the large intestine (34). From his idea evolved the idea of functional foods as a marketing term in the 1980s. This term is used to describe foods fortified with ingredients capable of producing health benefits. The trend for healthful foods is becoming increasingly popular with consumers because of the awareness that diet can influence health. Probiotic foods are defined as foods containing live microorganisms in significant concentrations that actively enhance the health of consumers by improving the balance of microflora in the gut when ingested (18). This restricts probiotics to products that contain live microorganisms, improve health and well-being of humans or animals, and can affect host mucosal surfaces including the mouth and gastrointestinal tract, the upper respiratory tract, or the urogenital tract (35). In Japan, a standard for probiotic foods was developed stipulating that a product contain greater than 1×10^7 viable *Bifidobacteria* per gram or milliliter of product (36). The probiotic must be viable and available at a high concentration to achieve health benefits (18). These factors are often overlooked or ignored, and many of the commercially available probiotic products have shown low populations of probiotics (37).

Fermented dairy products have evolved as the predominant carriers of probiotics in foods. They already have a healthful image that facilitates recommendations of daily consumption, and consumers are aware that fermented foods contain live microorganisms (38). Many of the fermented dairy foods have been optimized for survival of the fermentative microorganisms, which creates important technological advantages for the use of dairy products as probiotic carriers (39).

B. Health Issues

Foods that help prevent disease are becoming increasingly popular with consumers, with 70% of American shoppers believing that certain foods contain components that reduce the risk of diseases and improve long-term health (35). Additionally, 97% of shoppers surveyed in the United Kingdom were willing to change their eating habits to have an impact on their health. The purported benefits of probiotics include inflammatory disease control, the treatment and prevention of allergies, cancer prevention, immune stimulation, and respiratory disease reduction. Many of the suggested benefits of probiotic dairy foods are based on the involvement of the gastrointestinal microflora in resistance to disease. Unfortunately, few well-controlled studies have looked at clearly defined health effects, and many of the reported health benefits are based on unsubstantiated reports. However, the use of probiotics for the treatment of lactose maldigestion, diarrheal disease, lowering of serum cholesterol, and prevention of cancer or formation of carcinogens appear to be well substantiated (41). Lactose intolerance or maldigestion causing abdominal discomfort affects approximately 70% of the world's population to varying degrees (35). A probiotic bacterium, *Lactobacillus acidophilus*, has been clinically shown to alleviate the symptoms of lactose intolerance. The recent work in this area indicates that lactose in yogurt—possibly because of lactase-producing bacteria—can be utilized more efficiently than lactose in milk. Additionally, many of the probiotic strains that produce lactase have shown promise in alleviating symptoms of lactose intolerance (40).

Rotavirus is one of the leading causes of gastroenteritis worldwide (41). Gastroenteritis characterized by acute diarrhea and vomiting is a leading cause of death and illness among children, affecting approximately 16.5 million children annually (35). Ample evidence has shown that the probiotic strain *Lactobacillus GG* reduced the duration and severity of rotavirus infection. Oral administration of *Bifidobacterium bifidum* has shown potential for reducing the incidence of diarrhea in infants hospitalized for rotavirus infection

(41). Other research suggests that the probiotic bacteria *Bifidobacterium longum* and *Saccharomyces boulardii* prevent antibiotic-associated diarrhea (35).

Much attention has been given to the cholesterol-lowering potential of probiotics. However, the influence of lactic acid bacteria such as *Lactobacillus acidophilus*, although widely studied, remains controversial (42).

The role of lactic acid bacteria in reducing the incidence of DNA damage and other carcinogenic changes has also been investigated (43). In addition microflora and composition of the intestinal flora with probiotics might suppress the growth of bacteria that convert procarcinogens to carcinogens. An important index of carcinogenic activity is the activity of enzymes that convert procarcinogens to carcinogens. Several studies have shown an inhibitory effect on these enzyme activities following the consumption of probiotics (41).

Additional research on the prevention or delay of tumor development by probiotic bacteria suggests that they might bind to mutagenic compounds in the intestinal tract. A reduction of mutagens in urinary excretions was found when meals were supplemented with fermented milk containing *Lactobacillus acidophilus*. This suggests that lactobacilli were binding to the mutagenic compounds, thereby reducing their absorption in the intestine. It is inconclusive whether this will lead to a decreased incidence of cancer but research looks promising (41).

C. Probiotic Cultures

LB and ST are the bacteria traditionally used in yogurt manufacture. Although these bacteria have some health benefits, they do not naturally inhabit the gastrointestinal tract. They do not survive the acidic conditions of the stomach and the bile concentration of the GI tract (18). The probiotic bacteria used currently in commercial products include members of the genera *Lactobacillus* (Lb) and *Bifidobacterium* (B). The *Lactobacillus* (Lb) strains that are mainly used in commercial production include *Lb. acidophilus*, *Lb. johnsonii*, *Lb. casei*, *Lb. rhamnosus*, *Lb. gasseri*, *Lb. reuteri*, *Lb. helveticus*, and *Lb. plantarum*. *Bifidoacterium bifidum*, *B. longum*, *B. infantis*, *B. breve*, and *B. lactis* are some of the *Bifidobacterium* strains that are used commercially (38). Therefore, for a product to be considered a probiotic, many of these strains are incorporated into the fermented dairy product. Typically, combinations of these strains are used. A product manufactured entirely with probiotic bacteria would require a long incubation time and would lower taste quality; therefore, it is common practice to manufacture products with both yogurt and probiotic cultures (39).

D. Product Development and Production of Probiotic Dairy Foods

1. Food Interactions and Probiotics

There are several basic requirements for the development of marketable probiotic products. The most basic requirement is that the probiotic bacteria survive manufacturing and storage in sufficient number in the product so that they are viable when consumed. Additionally, the probiotic of choice should not have an adverse effect on the sensory properties of the food product. The chemical composition of the food product will also be important in determining the metabolic activity of the probiotic and how successfully it will survive. The amount and type of carbohydrate available, the degree of hydrolysis of milk proteins (amino acid content), and the composition and availability of short-chain fatty acids will be essential variables in developing a probiotic semisolid dairy food.

The interaction between the probiotic organisms and the starter organisms is another important factor to consider in the production of semisolid fermented dairy foods. Both synergistic and antagonistic effects between organisms are known to exist. This will require the identification of specific strains of probiotics that work in synergy with the starter organisms to achieve efficient acidification and multiplication of organisms during the fermentation process. Antagonistic effects that cause the production of undesirable compounds such as hydrogen peroxide, benzoic acid, and bigenic amines have been investigated and can be a limiting factor for combinations of starters and probiotics (38).

A critical factor in the development of probiotic dairy foods is the manufacturing procedure. When the probiotic is added following fermentation and before or after cooling, the interactions are kept to a minimum. The metabolic activity of both the starter organisms and the probiotic is drastically reduced at this point and will dramatically reduce interactions. It is critical to maintain cold storage of the product without fluctuations in temperature to maintain the quality of the finished product (38).

Another factor of considerable importance is the physiological state of the probiotic. When the probiotic culture is harvested, whether it is in the logarithmic or stationary phase of growth, and how it is handled after harvesting will be critical to the successful addition to a food product. Bacteria from the logarithmic phase have been shown to be much more susceptible to environmental stresses. Environmental conditions that signal to the bacteria the transition from the logarithmic phase to the stationary phase will have an effect on survival during the stationary phase.

2. Production of Semisolid Dairy Foods with Probiotic Properties

Because each semisolid dairy product is manufactured differently, there will be considerable variation with respect to starter culture, processing temperatures, ingredients, and fermentation time and temperature. All of these factors will influence the successful production of fermented dairy products containing probiotics. The methods used to manufacture stirred yogurt and yogurt drinks are best suited to probiotic incorporation following fermentation. Immediately before packaging, the probiotic can be stirred into the product. With set yogurts, the probiotic must be added during fermentation because stirring in the final stages of manufacturing would destroy the products' consistency (38). The chosen strain must first meet the technological and functional requirements and thus might compromise the full expression of the health properties.

The addition of probiotics to the manufacturing of cottage cheese can also vary. When the probiotic is added with the starter culture during fermentation, a high amount of the probiotic organism will be lost from the coagulum during draining of the whey. It is therefore very difficult to control the concentration of probiotics in the final product. During manufacturing, the whey-coagulum mixture temperature is raised to 50–55°C; this temperature can be destructive to the probiotic organisms. The addition of the probiotics with the cream and spices in the final stages of manufacturing offers the best chance of survival for the probiotic bacteria (38).

III. CONCLUSIONS

Despite considerable advances in scientific understanding of semisolid cultured dairy products, their manufacture involves fine art and skill. A full understanding of the complex interplay between biological, chemical, and technological entities is slowly emerging. Cul-

tured dairy products are different from other dairy products in that they contain deliberately added live lactic acid bacteria with or without other probiotic bacteria. Therefore, the choice of starter cultures and control of the fermentation process are key parameters when producing quality products. The demand for probiotics-based foods is growing. However, it can be expected that consumer confidence will decrease, unless the dairy industry offers products that have been scientifically proved to exhibit probiotic effects. Thus, additional research on the mechanisms by which probiotic cultures exert their effects on the host is required.

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7
Yogurt

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I. INTRODUCTION

The first time fermented milk (yogurt) was consumed is not known. Fermented milk foods are the predecessors of cheese, which originated about the time dairy animals were first domesticated, 8–10,000 B.C. (1).

Belief in the healthful aspects of yogurt for human beings has been noted in many civilizations for centuries. Elie (Ilya Ilitch) Metchnikoff was the director of the Pasteur Institute. He received the Nobel prize in medicine and physiology in 1908 for his classic work on phagocytes and phagocytosis, which formed a basis for the theory of immunity. He is credited for his studies on consumption of fermented milk and longevity. Metchnikoff was not the first to promote curdled milk such as yogurt. Hippocrates, in the 4th century B.C., in his "Application of Hygenic and Dietary Measures," spoke highly of yogurt (2). Leben, a fermented milk of the Middle East, is mentioned in the *Book of Job* (10:10), 1520 years before the birth of Christ (2).

In India, people have consumed yogurt, called dahi, for thousands of years. A significant amount of milk produced in India is consumed as yogurt. A few years ago, lassi, a sugared yogurt drink, was declared the state drink for the parliament in Punjab, a province in northern India. In different countries, fermented milk is called by different names. However, the word "yogurt" is gaining popularity. In the developing countries, yogurt is an indigenous milk product. In the peri-urban and rural areas of Delhi, India, fresh raw milk is available from small local dairy farms with 40–50 buffaloes (3). Most households make their own yogurt from morning and evening milk. Raw milk is boiled and allowed to cool. When it feels warm to the touch, it is inoculated from the previous day's fermentation. Some samples from these household fermentations have been examined in the author's laboratory. They contained mesophilic lactococci including diacetyl producers, thermophilic cocci, thermophilic lactobacilli, yeast, and some coliforms.

In the past 30 years, annual per capita yogurt consumption in the United States has grown six-fold to 2.31 kg (Table 1) (4). The consumption of fermented milk products (yogurt, sour cream, and buttermilk) in the United States is very low compared to other developed countries (Table 2) (5). Actual consumption of yogurt and related products in the United States is a bit higher than reported. Many ethnic groups that consume large quantities of plain yogurt ferment their own. There are a number of adults who do not eat yogurt because they do not tolerate lactose in milk. Incidence of lactose intolerance in

Table 1 Per Capita Consumption of Yogurt in the United States (kg)

1970	0.36	1980	1.13	1990	1.81
1971	0.50	1981	1.09	1991	1.91
1972	0.59	1982	1.18	1992	1.91
1973	0.64	1983	1.45	1993	1.95
1974	0.68	1984	1.63	1994	2.13
1975	0.91	1985	1.81	1995	2.31
1976	0.95	1986	1.91	1996	2.18
1977	1.04	1987	1.95	1997	2.31
1978	1.13	1988	2.04		
1979	1.09	1989	1.91		

Source: Ref. 4.

several ethnic groups is high (6). However, feeding trials with humans (7), and with rats (8), showed that yogurt containing viable microflora could be tolerated by individuals determined to be lactose intolerant. Other research workers demonstrated that the lactase (beta-galactosidase) activity of the yogurt culture remains in the intestinal tract after the culture has lysed and continues to break down lactose (9). This activity can be detected in duodenum and terminal ileum after consumption of viable yogurt (10).

The $2.9 billion yogurt industry is growing at about 3 to 4% every year. The segment catering to children under the age of 12 has shown huge gains. For example, Danimals, a yogurt product targeting children, has grown 137.9% (11). Excluding private label, the top nine refrigerated yogurt brands are dominated by Yoplait and Dannon. In a recent consumer survey of 40 yogurts, quality varied from flavor to flavor in a single brand (12). Many of the yogurts where characterized as astringent, thick, gummy, jam and candy-like flavors, chalky, and slightly bitter. Fruit-containing yogurts also contain large amounts of sugar. This is a huge problem for an estimated 16 million Americans with type 2 diabetes and another 20 to 30 million Americans with impaired glucose tolerance (13). Yogurt formulated for this segment of population can offer a big growth opportunity.

Table 2 Consumption of Fermented Milk Products Other than Cheese in Selected Countries

Country	1993 per capita (kg)[a]
Finland	38.02
Netherlands	29.64
Sweden	28.54
Iceland	25.85
Denmark	20.66
Switzerland	18.66
France	17.06
Federal Germany	14.77
United States of America	6.00[b]

Fermented milk products include yogurt, sour cream, and buttermilk.

[a] Adapted from Ref. 5.

[b] For 1998, Ref. 4.

This brief chapter on yogurt is written to bring a clear understanding of this simple product, which is now becoming a vehicle for introducing probiotics and prebiotics, believed to impart good health by maintaining a balanced and healthy gut. For more extensive discussion on yogurt, see Tamine and Robinson (5).

II. DEFINITION OF YOGURT

Yogurt is an acid gel made from the fermentation of a standardized milk mix by *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) (14). The solid-not-fat component of milk may be raised to 12% or higher to yield a custard-like gel. The fat in the product is adjusted to qualify for low-fat to fat-free yogurt.

A. Yogurt Description

Yogurt is the food produced by culturing one or more of the optional dairy ingredients with a characterizing bacterial culture that contains the lactic acid–producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. One or more of the other optional ingredients specified below may also be added before the culturing process. All ingredients used are safe and suitable. Yogurt, before the addition of bulky flavors, contains not less than 3.25% milkfat and not less than 8.25% milk solids-not-fat, and has a titratable acidity of not less than 0.9%, expressed as lactic acid. The food may be and shall be pasteurized or ultra-pasteurized prior to the addition of the bacterial culture. Flavoring ingredients may be added after pasteurization or ultra-pasteurization. To extend the shelf life of the food, yogurt may be heat-treated after culturing is completed, to destroy viable microorganisms.

Optional ingredients: Vitamins. (a) If added, vitamin A shall be present in such quantity that each 946 milliliters (quart) of the food contains not less than 2000 International Units (IUs) thereof, within limits of current good manufacturing practice. (b) If added, vitamin D shall be present in such quantity that each 946 milliliters (quart) of the food contains 400 IUs thereof, within limits of current good manufacturing practice. Optional dairy ingredients are cream, milk, partially skimmed milk, or skim milk, used alone or in combination.

Other Optional ingredients. (a) Concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, or whey modified by partial or complete removal of lactose and/or minerals, to increase the nonfat-solids content of the food; provided that the ratio of protein to total nonfat-solids of the food, and the protein efficiency ratio of all protein present, shall not be decreased as a result of adding such ingredients. (b) Nutritive carbohydrate sweetners. Sugar (sucrose), beet or cane; invert sugar (in paste or syrup form); brown sugar; refiner's syrup; molasses (other than blackstrap); high-fructose corn syrup; fructose; fructose syrup; maltose; maltose syrup, dried maltose syrup; malt extract, dried malt extract; malt syrup, dried malt syrup; honey; maple sugar; or any of the sweeteners listed in part 168 of this chapter, except table syrup. (c) Flavoring ingredients. (d) Color additives. (e) Stabilizers.

1. Lowfat Yogurt

Lowfat yogurt has a similar descriptions as given earlier, except the milkfat of the product before addition of bulky flavors is not less than 0.5% and not more than 2% (14).

2. Nonfat yogurt

Nonfat yogurt is the product as per the previous description of yogurt, except the milkfat content before the addition of bulky flavors is less than 0.5% (14).

B. National Yogurt Association (NYA) Criteria for Live and Active Culture Yogurt

According to the NYA (15), live and active culture yogurt is the food produced by culturing permitted dairy ingredients with a characterizing culture in accordance with the FDA standards of identity for yogurt. In addition to the use of ST and LB, live and active culture yogurt may contain other safe and suitable food-grade bacterial cultures.

Heat-treatment of live and active yogurt with description of yogurt with the intent to kill the culture is not consistent with the maintenance of live and active cultures in the product. Producers of live and active culture yogurt should ensure proper practices of distribution, code dates, and product handling conducive to the maintenance and activity of the culture in the product.

Live and active culture yogurt must satisfy the following requirements (15):

> The product must be fermented with both *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. The cultures must be active at the end of the stated shelf life as determined by the activity test described in item 3. Compliance with this requirement shall be determined by conducting an activity test on a representative sample of yogurt that has been stored at temperatures between 32 and 45°F for refrigerated cup yogurt. The activity test is carried out by pasteurizing 12% solids nonfat dry milk (NFDM) at 92°C (198°F) for 7 min, cooling to 110°F, adding 3% inoculum of the material under test, and fermenting at 110°F for 4 hr. The total organisms are to be enumerated in the test material both before and after fermentation by IDF methodology. The activity test is met if there is an increase of 1 log or more during fermentation. In the case of refrigerated cup yogurt, the total population of organisms in live and active culture yogurt must be at least 10^8/g cfu/g at the time of manufacture. It is anticipated that if proper distribution practices and handling instructions are followed, the total organisms in refrigerated cup live and active culture yogurt at the time of consumption will be at least 10^7 cfu/g.

C. Frozen Yogurt

Frozen yogurt resembles ice cream in its physical state. Both soft-serve and hard-frozen yogurts are popular. These are available in nonfat and lowfat varieties. These products are not very acidic. The industry standards require minimum titratable acidity of 0.30%, with a minimum contribution of 0.15% as a consequence of fermentation by yogurt bacteria (15,16). Technology for production of frozen yogurt involves limited fermentation in a single mix and arresting further acid development by rapid cooling, or a standardization of titratable acidity to a desirable level by blending plain yogurt with ice cream mix containing fruit/syrup base, stabilizers, and sugar and then freezing the mix in a conventional ice cream freezer. The mix is frozen at −6° and hardened at −40°C. The finished product pH may vary between 5.5 and 6.0, depending on consumer acceptance. Typical composition of nonfat hard-pack frozen yogurt may contain fat 0%, milk-solidnonfat 13%, sucrose 13%, corn syrup solids 36DE 6%, maltodextrin 10DE 2%, and stabilizer 1.2% (16). For further discussion see references (5,16,17).

III. YOGURT STARTER ORGANISMS

For manufacture of legal yogurt in the United States, *Streptococcus thermophilus* and *Lactabacillus delbrueckii* subsp. *bulgaricus* must be added for basic fermentation of the yogurt mix. Additional, optional organisms, preferably of human intestinal origin, may be incorporated in the yogurt either through the starter culture or blended in after fermentation is complete (14,15). The optional organisms may be selected from a long list of candidates (Table 3) (18,19). Some characteristics of organisms encountered in yogurts are listed (Table 4) (5,20,21).

A. *Streptococcus thermophilus*

This organism is a gram-positive, catalase-negative anaerobic coccus and it is largely used in the manufacture of hard cheese varieties, mozzarella, and yogurt. It does not grow at 10°C but grows well at 45°C. Most strains can survive 60°C for 30 min (22). It is very sensitive to antibiotics. Penicillin (0.005 IU/mL) can interfere with milk acidification (20). It grows well in milk and ferments lactose and sucrose. Two percent sodium chloride may prevent growth of many strains. These streptococci possess a weak proteolytic system. It is often combined with the more proteolytic lactobacilli in starter cultures. Most ST grow more readily in milk than lactococci and produce acid faster. These streptococci strains possess β-galactosidase (β-gal) and utilize only the glucose moiety of lactose and leave galactose in the medium (23).

The proteolytic activities of nine strains of ST and nine strains of LB cultures incubated in pasteurized reconstituted NFDM at 42°C as single and mixed cultures were studied (24). Lactobacilli were highly proteolytic (61.0 to 144.6 μg of tyrosine/mL of milk)

Table 3 Optional Cultures for Addition to Yogurt

Lactobacillus acidophilus
Lactobacillus rhamnosus
Lactobacillus reuteri
Lactobacillus casei
Lactobacillus gasseri
Lactobacillus johnsonii
Lactobacillus plantarum
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium adolescentis
Bifidobacterium infantis
Bifidobacterium lactis
Bifidobacterium longum
Enterococcus faecalis
Enterococcus faecium
Sacchromyces boulardi
Pediococcus acidilactici
Propionibacterium freudenreichii

Source: From Refs. 18 and 19.

Table 4 Selected Characteristics of Organisms Found in Yogurt

	% Lactic acid in milk	Lactic acid isomer	Growth		Carbohydrates fermented							
			15°C	45°C	Glucose	Inulin	Galactose	Fructose	Lactose	Sucrose	Ribose	Raffinose
Lb. delbrueckii sp. bulgaricus[a]	1.8	D	–	+	+		–	+	+	+	–	–
Lb. lactis[a]	1.8	D	–	+	+		[c]	+	+	+	+	–
Lb. helveticus[a]	3.0	DL	–	+	+		+	[c]	+	–	–	–
Lb. casei[b]	0.8	L	+	–	+		+	+	±	+	+	–
Lb. paracasei[b]		DL	+	[c]	+		+	+	+	+	+	–
Lb. rhamnosus[b]		DL	+	+	+		+	+	+	+	+	–
Lb. plantarum[b]		DL	+	–	+		[c]	+	+	+	+	+
Lb. acidophilus		DL	–	+	+		+	+	+	+	–	[c]
Streptococcus thermophilus[a]	0.7	L	–	+	+		–	+	+	+	–	–
B. bifidum		L	–	–	+	–	+	+	+	[c]	–	–
B. longum		L	–	–	+	–	+	+	+	+	+	+
B. infantis		L	–	–	+	[c]	+	+	+	+	+	+
B. breve		L	–	–	+	[c]	+	+	+	+	+	+
B. adolescentis		L	–	–	+	[c]	+	+	+	+	+	+

[a] Do not grow in 2.5% salt.
[b] Can grow in 6 to 8% salt.
[c] 11 to 89% of strains are positive.
Source: From Refs. 5, 20, and 21.

and ST were less protelytic (2.4 to 14.8 μg of tyrosine/mL of milk). Mixed cultures, with the exception of one combination, liberated more tyrosine (92.6 to 419.9 μg/mL) than the sum of the individual cultures. Mixed cultures also produced more acid (lower pH). Of 81 combinations of LB and ST cultures, only one combination was less proteolytic (92.6 μg tyrosine/mL) than the corresponding LB strain in pure culture (125 μg tyrosine/mL). *Streptococcus thermophilus* requires fewer amino acids than lactococci and lactobacilli. Only glutamine and glutamic acid, along with sulfur amino acids, are essential for all the strains that have been tested (25,26). Recently it was shown that ST possess a branched-chain amino acid (leucine, isoleucine, and valine) biosynthesis pathway as an essential pathway for optimal growth in milk. This pathway is thought to play a role in maintaining the internal pH of the organism by converting acetolactate to amino acids (26). These organisms also have urease, which produces ammonia from urea in milk, to counter the acid effects. Dairy lactococci do not have this pathway (26).

B. *Lactobacillus delbrueckii* subsp. *bulgaricus*

These organisms are gram-positive, catalase negative, anaerobic/aerotolerant homofermentative, and produce D-(-) lactate (1.8%) and hydrogen peroxide (27). These cultures have β-galactosidase activity; only the glucose moiety of lactose is utilized and galactose is released in the medium (28). LB has high level of protease activity in milk that reaches its maximum during the log phase; ST produces highly active peptidase instead of protease (29,30). In a recent study (30), it was demonstrated that LB reached maximum protease activity between 4 and 8 hr after incubation and then declined rapidly. When grown singly in pasteurized reconstituted NFDM at 42°C, ST and LB were less proteolytic compared to the mixed culture growth (24).

C. Associative Growth of ST and LB

A symbiotic relationship exists between ST and LB in mixed cultures (31); carbon dioxide, formate, peptides, and numerous amino acids liberated from casein are involved. Associative growth of rod and coccus results in greater acid production and flavor development than the single-culture growth (32,33). It has been established that numerous amino acids liberated from casein by proteases of LB stimulate growth of ST (34,35). In turn, ST produces CO_2 and formate, which stimulates LB (35–38). During the early part of the incubation, ST grows faster and removes excess oxygen and produces the stimulants noted above. After the growth of ST has slowed because of increasing concentrations of lactic acid, the more acid tolerant LB increases in numbers (22,29,39). For a one-to-one ratio of rod and coccus, inoculum level, time, and temperature of incubation must be controlled and bulk starter should be cooled promptly.

D. Bifidobacteria

This Y-shaped organism was isolated from infant stool in 1899 at the Pasteur Institute by Tissier (21). There are 24 species in this group. Nine of these are of human origin and the other 15 come from animals (40). These gram-positive organisms are strictly anaerobic. The degree of tolerance to oxygen depends on the species and the growth medium. It appears that the strains of *B. bifidus* are relatively aerotolerant (40). Optimum growth temperature for species of human origin is 36°–38°C; no growth occurs at 20°C. They do

not tolerate heat; *B. bifidus* is inactivated at 60°C. Two moles of glucose are fermented by the fructose-6-phosphate phosphoketolase pathway to 2 moles of L (+) lactate and 3 moles of acetate. Some formic acid and ethanol may also be produced. Bifidobacteria of human origin synthesize several vitamins, thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), folic acid (B_9), cyanocabalamin (B_{12}), and nicotinic acid (P) (40).

It has been observed that the number of bifidobacteria falls significantly in the stools of adults and the elderly. The proportions of various species of bifidobacteria vary with age of the humans. It appears that *B. infantis* and *B. breve* are favored in breast-fed infants and *B. adolescentis* predominates in bottle-fed infants and in adults (40). Allergic infants were reported to be colonized mainly by *B. adolescentis* species with a lower mucus-binding capacity than bifidobacteria from healthy infants (41). Bifidobacteria population may be reduced after the administration of a Western diet. All strains of bifidobacteria do not behave similarly. Morinaga Milk Industry Co, LTD, claims that their *B. bifidum* (BB536) is hardier and superior to other strains (42).

E. Starter Culture Propagation

These days, yogurt manufacturing plants are large and highly automated. Trouble-free functioning of these operations require predictable and dependable starter culture performance in the context of types of products and their sensory attributes. Following are some helpful hints.

1. Milk should be of good microbiological quality, free of antibiotics and inhibitors of bacterial origin.
2. Fresh milk heat-treated at 90 to 95°C for 5 min or 85°C for 30 min tends to give a balanced growth with a 1:1 or 2:1 ratio for ST:LB. When cultures are propagated in reconstituted NFDM, ST tend to show abnormally large cells within a chain. More severe heat treatment, such as autoclaving of milk, is somewhat inhibitory to ST and favors the growth of LB and can cause culture imbalance in favor of LB. It should be noted that ST first initiates growth followed by LB (22). A low population of ST will delay the completion of starter or yogurt fermentation. Heat-treatment at 90°C for a few minutes generally inactivates bacteriophages present in the milk.
3. Many yogurt producers prefer to use skim milk and condensed skim over nonfat dry milk to raise solids in the growth medium. Handling of nonfat dry milk is labor intensive and invariably results in dusting of plant equipment and overhead fixtures. Under these conditions, keeping the plant clean is difficult, and can result a higher incidence of yeast and mold. If nonfat dry milk is used, the area should be enclosed and isolated. The exhaust air should be filtered.
4. It is important to cool the starter when appropriate pH/% TA is attained. Higher acidity tends to reduce the ST count (22).
5. At any given time, the availability of phage-unrelated cultures suitable for specific yogurt attributes is rather small. These cultures should be handled carefully to grant them long life in the plant.
6. Monitor phage in the starter and in the environment on a regular basis.
7. Many of the defined cultures may contain up to six strains of lactobacilli. The culturing conditions should be carefully verified and controlled for uniform culture activity. If the starter contains probiotic cultures or other adjuncts, their numbers should be verified in the starter and product.

The plants should work closely with the culture suppliers for phage monitoring and culture performance.

F. Bulk Starter Preparation

This is one of the key operations and should be attended to carefully by trained personnel dedicated to this duty. The starter tank valves and pipes and hatch with gasket should be assembled and sterilized with live steam at low pressure (3 to 5 lb). Keep the bottom valve open for the condensate to drain. Continue to steam the tank for 30 min after the surface temperature in the tank has reached approximately 99°C. Turn the steam off and close the bottom valve.

Skim milk with total solids raised to 10–12% is either pumped cold and heated to 90°C and held for 60 min or the starter mix is heated to 90°C in a plate heat exchanger and then held at that temperature in the tank for 60 min. Some plants prefer to use reconstituted nonfat dry milk at 10–12% TS for their bulk starter. In certain operations, where large amounts of starter are used for yogurt inoculation, yogurt base is used for starter culture preparation. This brings ease of operation and eliminates yogurt composition variation. Cool the mix to 43°C. Close the chilled water valve early enough that the temperature does not fall below 43°C. For 500 gallons, thaw a can (350 ml) of frozen culture concentrate (10^{10} cfu/g) in 5 gallons of tepid water containing 100 ppm chlorine. Inoculate the tank, stir it for 5 min. Turn off the agitator and let it incubate quiescently for 6–8 hr to reach 0.9% titratable acidity. Cool the starter to 5°C, using slow agitation. Then turn off agitation. Turn on agitation for a few minutes before the starter is to be pumped. This rate of inoculation yields approximately 10^6 cfu/g of starter mix. For a healthy culture, it may take 8 to 10 hr to reach 0.9% acidity. Starter has $2–5 \times 10^8$ cfu/mL. Time to 0.9% titratable acidity also depends on the strain composition of frozen culture concentrate.

It is advisable to make a Gramstain of the fresh starter and run an activity test as described in Sec. II. B, using 1% inoculum. Such data are invaluable in tracking the performance of culture(s) and in preventing failed yogurt fermentation.

IV. YOGURT MIX PREPARATION

Fermented milk products have delicate flavor and aroma and require milk of good microbiological quality. A variety of yogurt mix can be formulated and standardized from whole milk, partially skimmed milk, condensed skim, nonfat dry milk, whey compositions, and cream. These mixes should be formulated to comply with regulations and meet consumer expectations. Clarified, fat-adjusted milk at 50°C should be blended with appropriate dry ingredients using a powder funnel. It should be allowed to circulate for a few minutes. The solids content of separated milk or whole milk can also be raised to 12% and 15%, respectively, by evaporation. The increased solids content prevents whey separation and improves the texture.

A. Sweeteners

Sweeteners may be added to yogurt as part of the mix before fermentation and/or through fruit preserved with sweeteners. Sucrose (sugar) is widely used in yogurt production. It provides a clean sweet taste that has no other taste or other odors. It complements flavors and contributes to desirable flavor blends (17). It can be used as a dry, granulated, free-flowing, crystalline form or as liquid syrup (67% sucrose). Inclusion of more than 5% sucrose in yogurt mix of 16–20% total solids may cause culture inhibition and lack of

flavor development (5,16). Several corn syrup preparations and other sweeteners are also available (5,17). Nonnutritive sweeteners such as Aspartame and Nutrasweet® are used in light products. These sweeteners have a lingering aftertaste (5) and have not fared well in consumer acceptance (12). Several newer sweeteners, Actilight®, Acesulfame-κ, Natren, Neohesperidine, and Thaumatin, are available. These could be used alone or in combination. The choice of sweetener(s) is determined by availability, cost, and its legal status for use in yogurt (5).

B. Stabilizers

The set yogurt gel structure results from an acid-casein interaction in which casein micelles at or near their isoelectric point flocculate and the colloidal calcium phosphate partially solubilizes as acidity increases. During the fermentation of milk, the pH gradually declines to around 4.5 and destabilized micelles aggregate into a three-dimensional network in which whey is entrapped (43,44). Appearance of whey on the surface of yogurt gel is due to syneresis, separation of serum from curd. In yogurt this defect is called wheying off (45).

In stirred-style yogurt, the three-dimensional network is disturbed when fruit and flavors are mixed into the plain yogurt. The texture and physical properties of the yogurt depend on the fruit, the stabilizer, and the rate of cooling (45).

Stabilizers are added to prevent surface appearance of whey and to improve and maintain body, texture, viscosity, and mouthfeel. Yogurt with lower milk solids have a greater tendency to synerese. Numerous stabilizers are available on the market. Generally a combination of several stabilizers is included in the formulation to avoid defects that may result from the use of a single stabilizer. A partial list of stabilizers used in yogurt include the following:

1. Gelatin is a protein of animal origin. It is derived from the hydrolysis of collagen. Only high-bloom gelatin should be used in yogurt making due to improved gelatin/casein interactions, its higher melting point, and its higher stabilizing ability (5). The term bloom refers to the gel strength. It disperses in cold but requires heat for activation. It is used at a 0.3–0.5% level. Microstructure of yogurt made with 0.5% gelatin under scanning electron microscopy did not show gelatin, and the structure did not differ from that of a plain unfortified yogurt. This yogurt was rated smooth in a sensory evaluation (46).
2. Whey protein concentrates are used at 1–2% of protein addition. In a study with skim milk yogurt fortified with dairy-based proteins, the yogurts made with casein-based products were coarser and inferior compared to those made with WPC at 1–1.5%. It was recommended that WPC should be used along with other stabilizers (47).
3. Gums are water soluble or dispersible polysaccharides and their derivatives. In general, they thicken or gel aqueous systems when used at low concentration. Gums are used to stabilize emulsion and prevent wheying off. Food gums are tasteless, odorless, colorless, and nontoxic (48). All are essentially noncaloric and are classified as soluble fiber. These are used at 0.2–1.5% depending on the application.

Locust beam gum is a seed gum. It has low cold water solubility. It is generally used where delayed viscosity development is needed. Dispersion of this gum when heated to about 185°F, and then allowed to cool, is high in viscosity. It works synergistically with carrageenan in some applications (48).

Guar gum is very similar to locust bean gum but is more soluble in cold water. It hydrates readily at pH 6–9. Its solubility is not affected by pH in 4.8 to 5.0 range. It does not cross-link well with carrageenan (48).

Carrageenan is derived from red sea weed. It is a mixture of various types, kappa, iota, and lambda. It may contain 60% of kappa form and 40% lambda. The kappa type forms a gel, whereas lambda does not. The polymer is stable at pH above 7.0 and has a tendency to degrade slightly at pH 5–7; it degrades rapidly below pH 5.0. The potassium salt of this gum is the best gel former, but the gels are brittle and prone to syneresis. This defect is prevented by the addition of a small amount of locust bean gum. It interacts with casein in milk and promotes stabilization of the yogurt gel (48).

Xanthan is produced by microbial fermentation. It is readily soluble in cold and hot water. It is not affected by pH changes. A synergistic increase in viscosity results from interaction of xanthan with κ-carrageenan and locust bean gum. These gels are prone to shear thinning. It also gives sheen to products, which may not be desirable in yogurt (48).

Protein, starch, modified starch, and tapioca-based starches can be used without affecting the flavor of yogurt. The stabilizer used in yogurt is generally a blend of stabilizers incorporated at 0.5 to 0.7% or less. The amount used also depends on milk solids level (5). A recent consumer survey of marketplace yogurts has indicated that many products are gummy, perhaps overstabilized (12).

C. Fruit and Fruit Flavorings for Yogurt

The growth and popularity of yogurt is largely due to fruit and sugar. For fruit-containing yogurt, the primary component of yogurt taste is the perceived degree of sweetness. This attribute of yogurt is believed to be responsible for its spectacular growth (49). In an earlier study, 79% of consumers preferred flavored yogurts (50). The surveys done in the United Kingdom indicated that 90% of yogurt sales were of fruit and flavored varieties (51).

Many fruit flavors, single or blended, are popular and these may vary with the season. Fruit preparations are added to make up 10 to 20% of the final product. A fruit preserve consists of 55% sugar and 45% fruit (16). These are cooked until the final solids reach 65–68%. The pH of these preparations is adjusted to 3.0–3.5 with citric acid or other food-grade acid. The processed fruit in most cases is filled aseptically in totes and shipped to yogurt plants. Transfer of fruit to yogurt should be done through sterile equipment to avoid yeast and mold contamination. The blending and the filler areas are very crucial to the microbiological quality of the yogurt with respect to yeast and mold. These areas should have HEPA-filtered air to keep out airborne yeast, mold, and other contaminants. Cardboard boxes should not be brought in these areas. Also, high-pressure water hoses should be avoided while fruit blending and packaging is going on.

D. Heat Treatment

The high-solids mix is given a higher heat-treatment than for conventional pasteurization. Generally, milk is heat-treated at 85–95°C and then held for 10–40 min. At these temperatures, bacteriophages and vegetative bacterial cells are inactivated and the growth of starter bacteria improves. Up to 60°C, there is no effect on whey protein. At 60° to 100°C, the whey proteins interact with each other and κ-casein. This interaction decreases dissociation of α_s- and β-casein and increases κ-casein dissociation (51). When skim milk was pre-heat-treated at 85°C for 30 min and 90°C for 2 min, whey protein denaturation

was 76.5% and 55.0%, respectively (52). This heat-treatment and resulting interactions increase the water-binding capacity of the protein system.

E. Homogenization

Homogenization of mix is carried out in two stages; first stage is at 10–20 mega-Pascal and the second stage is at 3.5. It reduces the fat globule size to less than 3 μm, which gives a rich mouthfeel. This prevents creaming of the mix upon storage. Homogenization also improves gel strenght upon fermentation due to greater protein-protein interaction.

V. YOGURT MIX INOCULATION AND INCUBATION

The inoculation rate may vary from 0.5 to 6%, depending on the type of yogurt and system set up. For yogurt fermented in the cup (set style), small surges of mix, 20–100 gal may be inoculated at 5% and packaged and then incubated at 43–45°C. Yogurt may reach pH of 4.7 to 4.8 in less than 2 hr and is then sent out of the hot room. If more time is available, the mix can be inoculated at 0.5 to 1.5%, which may take it 6 to 10 hr to reach pH 4.4 to 4.5.

The inoculation rate may vary from 0.5 to 6%, depending on the plant layout and equipment available. Two kinds of fruited yogurts are popular in the United States.

1. Set-style fruit-on-the-bottom. In this kind of yogurt, cups receive about 2 oz fruit preparation followed by inoculated mix at approximately 44°C in the filler room. The cups are placed in cases, and pallets are moved to the hot room maintained at approximately 48°C. The pH of product reaches 4.7 to 4.85 in approximately 100 min., then the yogurt is moved to the cooling tunnel. At the end of tunnel, the temperature of the yogurt is 16–18°C. It is then moved to the cooler.
2. Stirred-style yogurt or Swiss-style. In this type of product, fully fermented, plain yogurt at pH 4.3–4.4 is cooled to approximately 20°C and blended with the fruit preparation and filled in the cups. The palletized product is placed in the cooler. The texture and physical properties of the yogurt depend on the fruit, stabilizer, and the rate of cooling (45).

A. Yogurt Fermentation

Yogurt fermentation is a homolactic fermentation. Glucose metabolism by ST, LB, and *Lb. acidophilus* proceeds by the EMP pathway. Lactose utilization in ST, LB, and bifidobacteria involves lactose transport into the cells via cytoplasmic proteins (permeases). This translocation of lactose takes place without its chemical modification. This unphosphorylated lactose is hydrolyzed by β-galactosidase to glucose and galactose. Glucose is catabolized and galactose is secreted from the cells (23,53). The lactose permease is an active transport system, and the energy is provided in the form of a proton motive force developed by expulsion of protons. The excreted amount of galactose is proportional to the amount of lactose taken up. The current model for lactose transport in these bacteria is that a single transmembrane antiport permease simultaneously translocates lactose molecules into the cytoplasm and galactose out of the cell. The energy generated through galactose efflux thus supports lactose uptake into these cells. The average lactose content of yogurt mix of 13% milk solids-not-fat was about 8.5% (54). During fermentation it was

reduced to about 5.75%. The initial galactose content of the mix was a trace but increased to 1.20% during fermentation. Only a trace amount of glucose was noted (54). In commercial yogurts, lactose ranged from 3.31% to 4.74% and galactose varied from 1.48% to 2.50% (54).

There are a number of inhibitors for the yogurt culture that can impede or slow down lactose fermentation. Some of these are listed here; for further details, refer to (5,20,27,53). These are heat-sensitive lactenins, lactoperoxidase/thiocyanate/hydrogen peroxide (LPS), agglutinins, mastitic milk, antibiotic residues, hydrogen peroxide, detergents and santizer residues, and bacteriophages. Many of the inhibitors mentioned here may be seasonal and sporadic or accidental. Bacteriophages, on the other hand, are pernicious and can be devastating if not managed properly.

B. Bacteriophages

Bacteriophages (phages) are viruses that can infect bacteria and destroy one or more components of the yogurt culture. Phage are differentiated into virulent (lytic) and temperate phage, which reflect different growth responses in the bacterial host. Phage that infect and lyse the host cell are called virulent phage, whereas those that do not necessarily lyse their bacterial hosts, but instead insert their genome into the host cell chromosome, are called temperate phage. The propagation of virulent phage in the bacterial cell is called the lytic or vegetative cycle of phage multiplication and results in the release of new infectious phage progeny (55). Bacteriophages multiply much faster than the bacteria. A bacteriophage with a burst size of 100 can destroy a culture within a couple of generations. This can cause huge economic losses and result in inferior yogurt. Due to explosive growth of yogurt and mozzarella cheese production, a greater incidence of phage against ST has been reported (29). It is also noted that phage for lactobacilli appear less frequently (29,56). Recent work has shown that ST phages are closely related, at both the genetic and morphological level, making differentiation difficult. Electron microscopy studies revealed that both temperate and lytic phages were nearly identical, having small isometric heads and long noncontractile tails (57,58). Some lysogenic strains were autolytic at 45°C (58). New strategies to develop phage-resistant strains include antisense RNA technology and origin-conferred phage-encoded resistance (PER) (57).

Both lytic and temperate bacteriophages have been found in *Lactobacillus delbrueckii* subsp. bulgaricus and subsp. *lactis* (59,57). Strains of *Lactobacillus acidophilus* isolated from dairy products harbored temperate phages and some produced bacteriocins. One induced phage lysed nine other dairy lactobacilli including LB (60). Some of the LB were also sensitive to the bacteriocin produced by *Lb. acidophilus*.

Primary yogurt cultures, ST and LB should be carefully chosen and evaluated with respect to phage and compatibility with other adjuncts. Since many of the organisms used in yogurt may harbor temperate phage, propagated starter culture, yogurt, and environment should be monitored for phage. Use of aseptic techniques for propagation and production by properly trained personnel along with proper mix heat-treatment sufficient to kill phage are essential to keep phage under control.

VI. FLAVOR OF YOGURT

The flavor of yogurt depends on the milk, its heat-treatment, the starter strains used, the incubation temperature, and the balance of the organisms in the yogurt. Biochemically,

flavor compounds of yogurt include but are not limited to lactic acid, acetic acid, formic acid, propionic acid, butyric acid, acetaldehyde, acetone, diacetyl, acetoin, and several other compounds. In milk, ST produces formic, acetic, propionic, butyric, isovaleric and caproic acid, diacetyl, acetone, and some acetaldehyde. Lactobacilli, on the other hand, produce large quantity of lactic acid, acetaldehyde, diacetyl, peptides, and amino acids. Many of the compounds are derived from lactose and some from other components in milk (22). The flavor of yogurt can turn acidic and bitter during storage throughout shelf life. Protein degradation can continue during cold storage of yogurt and some peptides released may be bitter (22,61). Incubation temperature of yogurt below 30°C may also cause bitterness (61). It is again emphasized that the cultures used should be carefully selected to deliver quality attributes of yogurt throughout its shelf life.

VII. NUTRITIONAL AND HEALTH ASPECTS OF YOGURT

The nutritional value of yogurt is derived from milk. The value of milk and its products was recognized long ago. An example is a reference to the Promised Land as a land "flowing with milk and honey" (62). Yogurt has a higher nutrient density at 13–18% milk solids compared to milk at 12.3%. In literature, a number of healthful benefits have been assigned against various disease states (16,19,62). These disease states are colitis, constipation, various kinds of diarrhea, gastric acidity, gastroenteritis, indigestion, intoxication (bacterial toxins), diabetes, hypercholesteremia, kidney and bladder disorders, lactose intolerance, liver and bile disorders, obesity, skin disorders, tuberculosis, vaginitis and urinary tract infections, cancer prevention, prevention and treatment of *Helicobacter pylori* gastritis, and irritable bowel syndrome. Yogurt is a good source of calcium. One 8 oz serving will provide about 400 mg of calcium (62). It is recommended that milk and yogurt be consumed with oxalate-rich foods such as spinach and soy products. A diet of products rich in calcium decreases urinary oxalate and helps in the prevention of kidney stones (62).

In 1908, in an explanation of his longevity-without-aging theory, Metchinkoff stated that lactic acid bacteria in sour milk displaced toxin-producing bacteria, thus promoting health. At about the same time (1899), Tisser isolated bifidobacteria from the stools of infants and recommended administration of the same to infants suffering from diarrhea (63). Since that time, numerous studies on the use of lactic cultures in foods have continued. These studies have yielded variable results with regard to the health benefits of probiotics. In the past few years, the use of probiotics in yogurt and fermented products have exploded.

A. Definition of Probiotics

Probiotics are a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (64). Considerable work is going on in which combination of molecular biology techniques and phenotypical analysis is being used in deciphering the participation and the role played by the probiotics. This will certainly challenge our current concepts.

B. Benefits of Probiotics

Health benefits imparted by probiotic bacteria are strain-specific and not species-or genus-specific. The strains *Lactobacillus rhamnosus* GG (Valio), *Saccharomyces boulardii* (Bio-

codex), *Lactobacillus paracasei* Shirota (Yakult), and *Bifidobacterium* BB12 (Chr Hansen), have the strongest human health efficacy data, against some or all of lactose intolerance; rotaviral diarrhea; antibiotic-associated diarrhea and the associated *Clostridium difficile* diarrhea; and some other bacterial diarrheas and infections. Traveler's diarrhea may also be alleviated in some people by *Lactobacillus* GG. It is not yet possible to relate probiotic intake to prevention of bowel cancer in humans. However, there is stronger evidence for effects of the Shirota strain on bladder cancer in the Japanese population (65). Probiotics may also help in atopic eczema, irritable bowel syndrome, inflammatory bowel disease, *Helicobacter pylori* infections, and possibly in arthritic conditions (65). For early evaluation and comparison of probiotics in vitro, laboratory tests and standards for good clinical tests must be followed. These are listed in Tables 5 and 6. Over the years, many studies were done with different strains but there are only a few strains for which peer-reviewed published evidence from human clinical trials is available. The evidence from these trials is presented in Table 7 (65). In a 15-month-long study with human subjects consuming probiotic milk products containing *Lb. rhamnosus* DR20, it was observed that population of *Lactobacillus* and enterococcal content of the feces was transiently altered without markedly affecting biochemical or other bacteriological factors (66). Based on their work, the authors have proposed a new definition: "Probiotics contain microbial cells which transit the gastrointestinal tract and which in doing so, benefit the health of the consumer." This definition certainly calls into question some of the criteria set forth in Tables 5 and 6 for an organism to qualify as a probiotic.

It is generally agreed that *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, the yogurt bacteria, do not adhere to the mucosal surfaces of the intestinal tract during their transit through the gut (5,67,68). These organisms do not survive in the human gastrointestinal tract because of their low tolerance for bile salts (69,70). A combination of molecular methods have been used for the detection of lactic acid bacteria in the human gastrointestinal tract (41). *Lactobacillus delbrueckii* group was detected but not *Streptococcus thermophilus* (41). Recent feeding trials with Gottingen minipigs appear to indicate that these yogurt organisms do survive the passage to the terminal ileum (71). The numbers detected (10^6 to 10^7 cfu/g of chyme) are considered to be high enough for these organisms to be considered as potential probiotics. It is believed that the gut is home to 400–500 species of organisms. Recent studies have indicated that the

Table 5 In Vitro Tests for Probiotic Bacteria as Indicators of Human Health

Acid/pepsin (pH 2.0 for 105 min) and bile tolerance (physiological concentrations)
Fast growth rate and active metabolism
Acid production (rate of production, types of acids, and regio-specificity)
Production of bacteriocins
Immune response (local and systemic)
Vitamin production (e.g., folate, B group)
Colonization and adhesion (Caco2 and HT29 cells, mucus)
Antibiotic sensitivity patterns
Bile deconjugation
Inhibition of pathogens (e.g., *Salmonella typhimurium*, *Escherichia coli*, *Clostridium difficile*, *Clostridium perfringens*, *Candida albicans*)
Fecal enzyme concentrations (e.g., β-glucuronidase)
Safe history

Source: Ref. 65.

Table 6 Standards for Good Clinical Trials on Probiotics

Each strain identified by molecular methods and properties fully documented.
Extrapolation of data from strains of the same species is not acceptable.
Double-blind, placebo-controlled randomized studies, with a "crossover" design where possible.
Adequate number of treatments to avoid "confounding" of data by other ingredients and organisms.
Study end points should be unequivocally stated prior to commencement.
Currently acceptable statistical tests must be used.
Confirmation of findings by one or more independent research groups.
Publication in peer-reviewed journals of international standing.
Efficacy of the probiotic strain in different products should be assessed separately for each product against each health condition.

Source: Ref. 65.

total microbiota of each adult individual had a unique pattern reflecting differences in composition that are partly dependent on the host genotype (41). What happens to the balance of these in different human beings with different dietary habits and ages is not known. How the organisms become established in the gut in different segments is also not known. Much useful information is coming forth as this matter is studied seriously.

C. Prebiotic

Prebiotic is defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health (18). The beneficial effects of the presence of bifidobacteria in the gut are dependent on their viability and metabolic activity. Their growth

Table 7 Strains of Probiotic Bacteria for Which There Is Published Peer-reviewed Clinical Data (strains are listed in decreasing order of clinical evidence)

Strain	Conditions
Lactobacillus rhamnosus GG (Valio)	1, 2, 4, 5, 6, 7, 8, (12), 14, 15
Saccharomyces cereviseae Boulardii (Biocodex)	2, 3, 4, 5, 7, 11
Lactobacillus paracasei Shirota (Yakult)	2, 5, 6, 9, (10), 11, (12), 15
Bifidobacterium lactis BB12 (Chr Hansen)	1, 2, 3, 4, 5, 6, 11, 15
Lactobacillus reuterii (Biogaia)	1, 5, (10), (12)
Lactobacillus johnsonii La1 (Nestle)	6, 11, 14, 15
Enterococcus faecium SF68 (Cemelle)	2, 5, 10, (12), 13
Lactobacillus acidophilus La5 (Chr Hansen)[a]	2, 4, 5, 6, 11
Bifidobacterium longum BB536 (Morinaga)	2, 5?, 11, (12), (15)
Bifidobacterium breve (Yakult)	(1), 5
Lactobacillus acidophilus NFCM Rhodia USA)	1?, 5, 6, (12)
Lactobacillus plantarum 299v (Pro Viva, Sweden)	5, 13

Condition: 1, rotavirat diarrhea; 2, antibiotic-associated diarrhea; 3, *Clostridium difficile* pseudomembranous colitis; 4, traveler's diarrhea; 5, other acute bacterial diarrhea; 6, lactose intolerance; 7, bacterial vaginitis; 8, atopic eczema and food allergy; 9, bladder cancer; 10, elevated cholesterol; 11, chronic constipation; 12, bowel cancer; 13, irritable bowel syndrome; 14, *Helicobacter pylori*; 15, immune response modulation. ?, doubtful evidence; (), animal data and/or biomarkers only.

[a] Data for this strain uncertain because it was coadministered with *B. lactis* BB12 usually.

Source: Ref. 65.

is stimulated by the presence of complex carbohydrates known as oligosaccharides. Some of these are considered prebiotics (72). Fructo-oligosaccharides (FOS) are well-known prebiotics that are found in 36,000 plants (73). FOS may contain two to eight units in a chain. Inulin, a type of FOS extracted from chicory root, has a DP (degree of polymerization) up to 60. FOS and inulin occur naturally in a variety of fruits, vegetables, and grains, especially chicory, Jerusalem artichoke, bananas, onion, garlic, asparagus, barley, wheat, and tomatoes (73). Recommended level of prebiotics begin at 5 g/day (73) and may be as high as 15–20 g/day (18).

1. Benefits of Prebiotics

Prebiotic fermentation leads to health benefits such as increased fecal biomass and increased stool weight and/or frequency. Prebiotics are fermented by bifidobacteria with the production of short chain fatty acids (SCFA), mainly acetate, propionate, butyrate, hydrogen, and carbon dioxide. Production of SCFA leads to lower pH in the colon, which facilitates absorption of calcium, magnesium, and zinc (18,73). Lower pH also restricts pathogenic and other harmful bacteria, thus reducing or eliminating precarcinogenic activity (73).

2. Potential Prebiotics

Several materials are under study as prebiotics: oligosaccharide (FOS Rafitilose P95), inulin, pyrodextrine, galacto-oligosaccharides, soy-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, lactulose, transoligosaccharides, Rafitaline, HP, Frutafit EXL, and Fruitofit IQ (73,74). In a recent study of 54 strains of lactic acid bacteria, lactose-derived galacto-oligosaccharide utilization was linked to the presence of β-galactosidase in bifidobacteria studies (75).

3. Synbiotic

Synbiotic refers to a product in which a probiotic and prebiotic are combined. The synbiotic effect may be directed toward two different regions, both the large and small intestines. The combination of pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own (76).

4. Survival of Probiotics in Yogurt

It is known that bifidobacteria and lactobacilli are members of the human gut flora. Also, a large number of yogurt brands claim the addition of probiotics. For the probiotics to be effective, the organisms have to be alive (19). In market-bought samples, the fresh product had 10^6 to 10^7 cfu/g. The numbers declined to less than 10^3 cfu/g in some products (77,78). Some strategies to improve probiotics numbers in yogurt include reduction of regular ST and LB in the product, and the addition of cultured probiotics to yogurt (78,79). Because the dietary probiotics appear to be transitory, feeding of probiotics, inulin and FOS, may be sufficient to favor the growth of healthy organisms in the gut (80).

VIII. FUTURE TRENDS

The market for probiotic drinks is booming, and clinical work with probiotics continues to provide support for their benefits. Yakult, from Japan, is the world's largest probiotic dairy beverage, followed by Actimel from Danone (81). The dividing line between yogurt

and cultured milks is becoming thinner. Probiotic strains may become the sole fermenting agents in yogurt and fermented milk (82,83). Biogaia and Farm Produce Marketing Ltd. have signed an agreement to market and sell drinkable yogurt with Biogaia's delivery system Life Top™ straw. The straw will be attached to the side of the package and contains *Lactobacillus reuteri* (Reuteri TM) (84). Under the category of healthy and functional yogurts, new fruits and flavors are being offered (85). Some of these are:

Yogurt containing fruit pieces and flavors such as melon and pink grapefruit
Yogurt with herbal extracts, ginger, green tea extract
Yogurt with passionfruit enhanced with elderflower extract
Strawberry/rhubarb fitness yogurt fortified with vitamins B, C, and E

There is a growing interest in offering premium and self-indulgent yogurts as desserts (85). Some of the creations listed include:

Bourbon vanilla
Pear and butterscotch
Goat yogurts containing mandarin and ginger, lemon and lime zest, and summer berries

We have come a long way in the development of yogurt into a respectable dairy product with noted health benefits. Producers and marketers of this product are making every effort to keep the yogurt category growing through product development and packaging innovations while delivering a good-for-you flavorful product suited for all occasions of gastronomic indulgence.

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8
Sour Cream and Crème Fraîche

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I. INTRODUCTION

Sour cream is a relatively heavy, viscous product with a glossy sheen. It has a delicate, lactic acid taste with a balanced, pleasant, buttery-like (diacetyl) aroma (1). Various types of sour cream are found in many regions of the world. The products vary in regard to fat content and by the presence or absence of nondairy ingredients. Furthermore, both cultured and direct acidification is utilized to lower pH. This chapter will cover sour cream as it is produced in the United States and its French counterpart—crème fraîche.

II. SOUR CREAM

A. Definition

The U.S. Food and Drug Administration (21CFR 131.160) defines sour cream as follows (2): "Sour cream results from the souring, by lactic acid producing bacteria, of pasteurized cream. Sour cream contains not less than 18 percent milkfat; ..... Sour cream has a titratable acidity of not less than 0.5 percent, calculated as lactic acid." If stabilizers are used, the fat content of the dairy fraction must be at least 18% fat and above 14.4% of the entire product.

Consumer desire for decreasing dietary fat content has created a market for low-fat sour creams. Among these products, the reduced fat (at least 50% fat reduction), and nonfat versions are common, in part due to FDA labeling requirements for low-fat products (21 CFR 101). Sales data over the past 25 years for the U.S. market (3) is illustrated in Fig. 1. The trend clearly shows increased sales. In 2000, nearly 400 million kg of sour cream was sold. Per capita sales of sour cream and dips was 1.4 kg. In comparison, per capita sales for yogurt, heavy cream, and half and half were 2.1 kg, 0.9 kg, and 1.7 kg, respectively (3).

B. Sensory Characteristics

Traditionally, the flavor of sour cream was well characterized by "sour." However, the trend in cultured dairy products is toward a milder flavor (4), which permits the sensation of aromatic compounds produced by lactic acid cultures. Lindsay et al. (5) found that important flavor compounds in sour cream include diacetyl, acetic acid, acetaldehyde, and

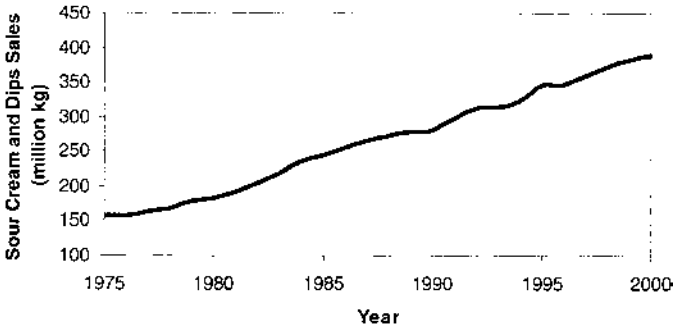

Figure 1 Sales, in million kg, of sour cream and dips in the United States between 1975 and 2000. From USDA, Agricultural Marketing Service.

dimethyl sulfide. Sour cream is highly viscous and should be smooth and free of particulate matter. As for appearance, a homogeneous, glossy surface is preferred, and no whey separation should be visible in the container (6).

C. Utilization

Sour cream is predominantly utilized as an accompaniment with warm entrees such as baked potatoes and burritos. This usage imposes certain demands on the sensory characteristics of the product, especially in regard to texture when in contact with warm surfaces. Sour cream must remain viscous without whey separation when placed on warm food. Some have even requested that baked potatoes can be reheated in the microwave with sour cream already added, and the sour cream should remain unaltered by this treatment. In addition, flavor characteristics become less significant when sour cream is mixed with high-intensity savory flavor notes such as those encountered in Mexican cuisine. In fact, for some usages the absence of off-flavors may be considered as the primary flavor attribute. This general shift in emphasis away from flavor toward texture has led to a renewed interest in a "back to basics" sour cream such as crème fraîche, which is described later in this chapter.

III. FERMENTATION

As with all fermented dairy products, the choice of starter culture is crucial for the production of high-quality sour cream (7). Mixed strains of mesophilic lactic acid bacteria are used for sour cream. In general, both acid and aroma producers are utilized. Acid producers include *Lactococcus (Lc) lactis* subsp *lactic* and *Lc lactis* subsp *cremoris*. *Lc lactis* subsp *lactis* biovar *diacetylactis* (or *Cit*$^+$ *Lactococci*) and *Leuconostoc mesenteroides* subsp *cremoris* are commonly used aroma producers.

The acid producers convert lactose into L-lactate through a homofermentative pathway. They can produce up to 0.8% lactic acid in milk (8) and are responsible for lowering pH in the fermented product.

In contrast, aroma producers are heterofermentative and can convert lactose into D-lactate, ethanol, acetate, and CO_2. In addition, these strains convert citrate into diacetyl, which is one of the major flavor compounds responsible for typical sour cream flavor.

Diacetyl is subsequently partially converted into acetoin, which is a flavorless compound (9). Extensive research at starter culture companies has led to the development of *Leuconostoc* strains that show less of a tendency to convert diacetyl into acetoin, thus retaining high levels of diacetyl (D. Winters, personal communication, 2002). Use of such strains can extend the shelf life of sour cream because it takes longer for the product to turn stale. *Leuconostocs* also reduces acetaldehyde to ethanol (10,11). In fact, acetaldehyde has been shown to promote the growth of *Leuconostoc mesenteroides* subsp *cremoris* (12,13). Acetaldehyde is typically assocaited with yogurt flavor (green apple) but is considered an off-flavor in sour cream.

The choice of starter cultures will affect product texture as well. Strains of acid producers have been developed that increase viscosity through the production of exopolysaccharides (14). These polysaccharide chains contain galactose, glucose, fructose, mannose, and other sugars. Quantity and type depend on the bacteria strain and growth conditions (15,16). The exopolysaccharides interact with the protein matrix creating a firmer network and increasing water-binding capacity. The importance of this behavior was confirmed by Adapa and Schmidt (17), who found that low-fat sour cream, fermented by exopolysaccharide-producing lactic acid bacteria, was less susceptible to syneresis and had a higher viscosity.

Production of high-quality sour cream requires a fine balance of acid-, viscosity-, and flavor-producing bacteria. This balance varies among commercially available strains, but a typical combination would be 60% acid producers, 25% acid and viscosity producers, and 15% flavor producers (D. Winters, personal communication, 2002).

IV. GEL FORMATION

Fermentation leads to a significant increase in viscosity. Two physicochemical changes cause this behavior (18,19). The casein submicelles disaggregate because of solubilization of colloidal calcium phosphate. In addition, the negative surface charge on the casein micelles decreases as pH approaches the isoelectric point. This creates the opportunity for casein micelles to enter into a more ordered system. Besides the protein network, cream gains viscosity from the formation of homogenization clusters (20). Following single-stage homogenization at room temperature, milk fat globules will cluster, and these clusters may contain up to about 10^5 globules (21). Casein molecules adsorb onto newly formed fat globule membranes and, in the case of high fat content, form bridges between fat globules. Clustering increases viscosity because (a) serum is entrapped between the globules and (b) irregularly shaped cluster are formed.

V. STABILIZERS

The gel structure may not be sufficiently firm to withstand abuse during transportation, handling, and storage. This could result in a weak-bodied sour cream and whey syneresis in the container. These defects are especially noticeable for low-fat products. To ensure consistent firm texture, dairy processors often choose to add nondairy stabilizers (22). Stabilizers commonly found in sour cream include polysaccharides and gelatin.

Stabilizer must be food grade and approved. The type and quantity used vary widely dependent on fat content, starter culture, and required sensory characteristics of the final product. Types and quantities of potential stabilizer mixtures used in sour cream are

Table 1 Examples of Stabilizer and Other Ingredients Used in Sour Cream

Product	Ingredients	Usage level
Sour cream	Modified food starch, grade A whey, sodium phosphate, guar gum, sodium citrate, calcium sulfate, carrageenan, locust bean gum	1.5–1.8%
Low-fat sour cream	Same as above	1.75–2.0%
Nonfat sour cream	Modified food starch, microcrystalline cellulose, propylene glycol monoester, gum arabic, artificial color, cellulose gum	6.2–6.6%

Source: From Ref. 27.

outlined in Table 1. Especially, the nonfat formulation contains other ingredients such as emulsifiers, color, and protein.

Polysaccharides bind water and increase viscosity. Commonly used plant polysaccharides include carrageenans, guar gums, and cellulose derivatives. Modified starches are frequently utilized as well. It is necessary to fully hydrate these polysaccharides to optimize their functionality. Depending on the ingredient, this may require efficient blending systems for incorporation of the ingredient into the cream, though care should be taken to avoid churning the cream. Complete hydration can sometimes only be accomplished following heating and cooling steps, which conveniently are done by the pasteurization process. Time may also be a factor for hydration to occur. Besides binding with water molecules, polysaccharides may also interact with milk proteins and form a network, which limits the movement of water and increases viscosity. A short description of the stabilizers is provided below:

Carrageenans. Extract of seaweed. Three types of carrageenans are commercially available, lambda, iota, and kappa, which differ based on the amount of sulfate. They have low viscosity at high temperature but viscosity increases during cooling. Lambda has the highest sulfate content, is soluble in cold milk, and forms weak gels. Iota is soluble in hot milk (55°C) and prevents syneresis. Kappa dissolves only in hot milk (>70°C) and forms brittle gels (23).

Guar gum. Endosperm of seed from Cyanopsis tetragonolobus plant. Different types of guar gum are available to fit processing conditions. Maximum viscosity develops over time. All are soluble in cold milk. The main component is mannose with attached galactose units.

Methylcellulose. A cellulose that improves freeze-thaw stability and prevents melt upon heating (22).

Gelatin. In contrast to the polysaccharides described above, gelatin consists primarily of protein (84–86%) and is derived from animal sources such as skin and bones (24). Gelatin is an excellent gelling agent but some off-flavors are perceived when used at excessive concentrations.

VI. PROCESSING

Throughout the processing of sour cream, extra care should be taken to protect the cream. Prior to pasteurization, rough cream treatment could lead to rancid off-flavors due to

lipolysis. Following fermentation, it is important to treat the coagulum gently to retain body and texture. This includes use of positive-displacement pumps instead of centrifugal pumps, round pipe elbows instead of 90° angles, and use of gravity feed wherever possible. In addition, special cream pasteurizers may be used. A process flow chart of a typical sour cream process is shown in Fig. 2.

Ingredients can be incorporated directly into standardized cream by mixing equipment such as a triblender. Another option is to incorporate the dry ingredients into the milk portion before standardizing the cream. The mix is preheated and homogenized (~65°C, 10–25 MPa) (25,26) immediately prior to pasteurization. Dairy homogenizers are normally double-stage to prevent homogenization clusters. However, in sour cream production single-stage homogenization is preferred to build up body of the product. Additional vis-

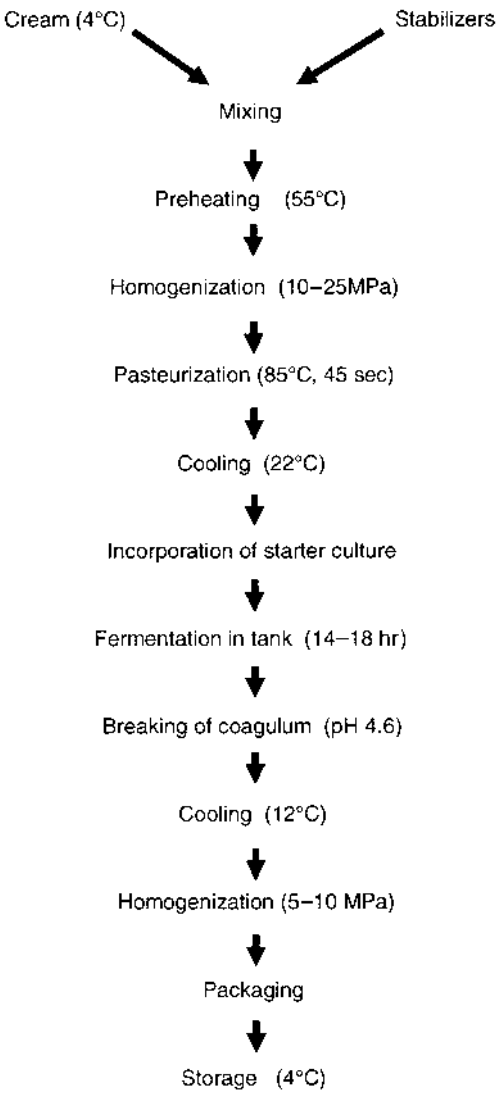

Figure 2 Process flow chart of typical sour cream process.

cosity is obtained if the cream is homogenized downstream from the pasteurizer, though such a process increases the potential for postpasteurization contamination. Pasteurization is done at relatively high temperatures (85–90°C for 10–45 sec), well above what is required for destruction of pathogens. The more severe heat treatment lowers the potential for oxidative and rancid off-flavors during storage and may help improve product viscosity as well. The cream is cooled to 22–25°C, pumped into the fermentation tank, and starter culture is added. Gentle mixing should continue until culture and cream are properly mixed (maximum 30 min). At this point, mixing is stopped until fermentation is complete. The fermentation tank may be double-jacketed to allow for better temperature control. However, in reality this is not essential if the temperature of the processing room remains relatively constant around 22°C. Fermentation temperature may vary slightly from plant to plant. Higher temperatures lead to faster fermentation and potentially a more acidic product, whereas lower fermentation temperatures may give a more flavorful product. The fermentation is slowed down/stopped by cooling when the desired acidity (~pH 4.5 or titratable acidity around 0.7%–0.8%) is achieved. Typically this takes 14–18 hr. The coagulum is broken by gentle stirring and the product is cooled either by pumping cooling water into the double-jacketed area of the tank or by pumping the cream through a special plate cooler. The cream should be cooled to around 8–12°C, which slows starter culture activity before packaging. Prior to packaging, it can also be passed through a homogenizer screen (smoothing plug) or similar type of flow restrictor to smooth and improve texture (27). The final cooling to around 4°C must occur slowly in the package in the cooler in order to allow the cream to obtain the appropriate viscosity. It is essential that the cream not be moved during this cooling step.

The above process assumes large-scale production. However, numerous process variations exist, as follows.

A. "Short Cuts"

Throughout the process described above, special attention is focused on gentle treatment of the product to assure proper body and texture. In reality, the stabilizers used today permit more flexibility in the process. A certain amount of product abuse can be tolerated without lowering the product quality because the stabilizers, when properly used, create a firm texture and prevent whey separation.

B. Low Quantity

It is possible to significantly simplify the process when producing small quantities of product. Sour cream can be made with a double-jacketed pasteurization tank, a pump, and a fermentation tank with gravity feed to the filler. The absence of a final in-line cooling step would require an efficient cooling procedure for the packaged product.

C. Chymosin Addition

Low quantities of chymosin may be added at the same time as the starter culture. This creates a more "spoonable" sour cream. Lee and White (28) found that chymosin addition (e.g., 0.066 ml/L) to low-fat sour cream resulted in increased viscosity and whey separation. Sensory scores were lower for the chymosin-containing sour cream in regard to flavor, body/texture, and appearance. This indicates that it may be preferable to modify the stabilizer mixture rather than to add chymosin when trying to increase product viscosity.

D. Set Sour Cream

The standardized, pasteurized cream can be mixed with starter culture and immediately filled into the package. The cream is then fermented within the final package, which leaves the coagulum undisturbed. When the appropriate acidity is obtained, the products are cooled either by passing through a blast cooler or by placement in a cooler. The advantage of this method is the possibility of lowering or eliminating stabilizers and yet obtaining excellent body and texture. The disadvantages are the large space requirement for fermenting the packaged product and the relatively slow cooling.

E. Direct Acidification

A product somewhat similar to sour cream can be obtained by direct acidification by organic acids such as lactic acid, instead of fermentation. However, Kwan et al. (29) and Hempenius et al. (30) found that sensory panelists preferred cultured sour cream instead of chemically acidified cream. Product temperature at the time of acidification is critical and should be around 20–25°C. Higher temperatures increase the likelihood that graininess occurs, and lower temperatures increase the time required for gel formation (27).

F. Low-Fat and Nonfat Sour Cream

Vitamin A fortification is required in these products. The processes are often similar to traditional sour cream though nonfat sour cream mix should be homogenized at much lower pressure. The main difference is observed in the stabilizer mix as described above in Sec. V.

VII. SHELF LIFE

Sour cream should have a shelf-life of around 25–45 days. One study documents that when properly stored undisturbed at 4°C, sour cream has an acceptable shelf life for up to 6 weeks (31). In another study, Folkenberg and Skriver (7) evaluated the change of sensory properties of sour cream during storage time. As storage time approached 28 days, the intensity of prickling mouthfeel, sour odor, and bitter taste increased. The samples were stored under ideal conditions, which suggest that real life distribution and storage temperature abuse would likely decrease the shelf life of this product below 28 days.

The single most important factor determining shelf life remains cream quality. Unless the cream is of excellent quality, the sour cream quickly develops off-flavors. Two parameters that impact cream quality are (a) raw milk quality and (b) pretreatment of milk. Good-quality raw milk has a low bacterial content (low standard plate count) and comes from healthy cows (low somatic cell count). Even good-quality raw milk spoils unless quickly cooled and kept at low temperatures until pasteurization. Furthermore, the time interval between milking and pasteurization should be as short as possible. Other factors to consider are proper cleaning and sanitation of all milk contact surfaces, well installed and sized pumps, and no unnecessary milk handling.

Assuming that high-quality cream is utilized, the parameters that limit shelf life tend to be associated with either flavor defects or surface growth of yeast and molds. When using appropriate stabilizers, the body and texture should remain adequate throughout the shelf life. A guide on how to prevent flavor defects is included below. Yeasts and molds are controlled by improving sanitation throughout the process. As with many other dairy

products, sanitation trouble spots are often associated with the filler machines, which are difficult to clean.

VIII. SENSORY DEFECTS IN SOUR CREAM

A. Flavor

The high lipid content makes sour cream extremely vulnerable to lipid-associated off-flavors such as rancidity and oxidation. Other flavor defects include flat taste, lack of cultured flavor, and high acid.

1. Rancid

Hydrolytic rancidity or lipolysis is caused by the release of free fatty acids from the glycerol backbone of triglycerides. The reaction is catalyzed by the lipase enzyme, which can be a native milk lipoprotein lipase or can originate from bacterial sources. Triglycerides are generally protected from lipase activity as long as the milk-fat globule remains intact. However, damage to the globule will lead to rapid lipolysis because lipase, which is situated on the surface of the globule, can access the triglycerides. Therefore, precautions must be taken to prevent damage to the milkfat globule until pasteurization, which denatures most types of lipase. This means that raw milk/cream must be pasteurized before or immediately after homogenization to assure denaturation of lipase. Likewise, it is strongly recommended never to recycle pasteurized milk/cream back into raw milk/cream storage, which is essentially an issue of rework handling. Cream from poor-quality raw milk can also develop rancid off-flavors during storage because some bacterial lipases are quite heat stable and do not denature during pasteurization.

2. Oxidized

Autoxidation of milk fat is a reaction with oxygen that proceeds through a free radical mechanism. Unsaturated fatty acids and phospholipids are the prime substrates that are broken down into smaller-molecular-weight compounds such as aldehydes and ketones. Oxidized cream exhibits off-flavors and aromas that have been characterized as cardboardy, metallic, oily, painty, fishy, and tallowy (6). Oxidation is catalyzed by divalent cations such as iron or copper. Thus, the best prevention is to avoid contact of milk/cream with these metals. This requires attention to details, as a single fitting or pipe made of these metals can cause significant autoxidation.

3. Lacks Fine Flavor/Lacks Cultured Flavor

Both flavor defects tend to be associated with the choice of starter culture. It may be possible to improve flavor by switching to culture systems with more aroma-producing capacity or to strains that retard the transfer of diacetyl into acetoin. It is also possible to add low concentrations of citric acid (below 0.1%), which is then converted to diacetyl by the aroma-producing starter cultures. The defect can also result from flavors imparted by stabilizers. Lowering the stabilizer dose or changing to another stabilizer system may be required.

4. High Acid

If the final product pH is very low (e.g., around pH 4.0), the product has an unpleasant sour flavor. Although it is possible to stop the fermentation at a higher pH, this does not necessarily solve the problem because slow fermentation continues in the cooled and

packaged product. Therefore, it is often preferable to change the starter culture mixture to lower the ratio of acid-producing bacteria.

5. Bitter

Bitter off-flavors are often indicators of excess proteolytic activity. Poor-quality raw milk may contain heat-stable proteases that remain active throughout storage. The defect is especially noticeable at the end of shelf life. Improving raw milk quality, increasing pasteurization temperature, or shortening code dates are possible solutions.

B. Body and Texture

As described above, texture is an essential quality parameter. Sour cream must remain highly viscous when in contact with warm food surfaces such as baked potatoes.

1. Too Firm or Weak

Improper choice of stabilizers can cause overstabilized sour cream that clings to the spoon. Alternatively, the sour cream can be weak-bodied and "melt" on the hot food surface.

2. Grainy

Grainy is primarily a mouthfeel problem, though it can be visually distracting as well in extreme cases. Grainy sour cream can be an indication of poor blending or incomplete hydration of ingredients. A different choice of stabilizers or a modification of incorporation procedure may improve the product. Another solution is to pass the product through a single-stage homogenizer valve prior to packaging. Grains can also indicate that the fermentation was stopped at too high a pH and the caseins are at their isoelectric point, around pH 4.6.

3. Free Whey

Whey syneresis on top of the sour cream in the package is considered a significant quality defect. There are three solutions available for solving the problem.

1. Change or increase the concentration of stabilizer.
2. Increase fat content. Higher fat sour creams have a better water-binding capacity.
3. Reevaluate the entire process and eliminate points of product abuse. This would primarily include all steps after fermentation.

IX. CRÈME FRAÎCHE

Crème fraîche, or more correctly crème fraîche épaisse fermentée, is the European counterpart to the U.S. sour cream product. Crème fraîche has a fat content around 30–45% and has a mild, aromatic cream flavor. The differences between the two products originate in the manner of usage. The usage of sour cream is described above. Crème fraîche is used cold on desserts such as fruit or cakes, or warm as foundation in cream sauces that are commonly used in the French cuisine. This double usage creates a unique demand for specific product attributes. The dessert utilization requires a clean, not too sour (4), cultured flavor that doesn't overpower flavors from other dessert components. The cultured flavor should be refreshing so that it covers the impression of fat in the product. This emphasis on flavor has

led to significant research at starter culture companies and dairy processing companies to develop starter cultures that cause optimum flavor development. The body and texture should be smooth and less firm than sour cream. Crème fraîche should be "spoonable", not "pourable", and should spread slightly on the dessert without being a sauce.

The incorporation of crème fraîche into warm sauce requires thermostability, otherwise the protein would precipitate and flocculate in the sauce. For regular crème fraîche (>30% fat) flocculation is rarely a problem. In contrast, low-fat crème fraîche (~15% fat) is less stable when heated. Addition of stabilizers such as xanthan gum can stabilize low-fat crème fraîche. However, based on European labeling legislation, a crème fraîche can not contain stabilizers and a stabilized product would therefore need to be marketed under another name.

Crème fraîche is produced by a process similar to that of sour cream, with the exception that no ingredients are added. Without stabilizers, it becomes a challenge to obtain good body and texture. Each processing step requires attention to producing and maintaining high viscosity. In this case the homogenizer becomes an essential tool for building viscosity. Only single-stage homogenization is utilized. The product is sometimes homogenized twice, either in subsequent runs before pasteurization, but more commonly both before and after pasteurization.

Homogenization after pasteurization promotes better viscosity and, equally important, better thermostability. An additional homogenization following fermentation gives a homogeneous product with a smooth mouthfeel. Homogenization downstream from the pasteurizer (i.e., after pasteurization) should raise concerns in regard to post-pasteurization contamination. Ideally, an aseptic homogenizer should be used. However, the high price of such homogenizers makes this an unsuitable alternative. Instead, great emphasis must be placed on proper cleaning and sanitizing of the downstream homogenizer. In addition, food safety issues are normally controlled because of the high content of lactic acid bacteria and the low pH.

There is some discussion as to the final pH of crème fraîche fermentée. Kosikowski et al. (25) and Kurmann et al. (32) state that the cream is fermented to pH 6.2–6.3. However, commercially it is commonly fermented to an end pH around 4.5. The mild flavor is not obtained by a higher pH but rather through selection of aroma-producing starter cultures. It is the combination of aroma compounds and the high fat content that mask the sour flavor in crème fraîche.

Crème fraîche is a new product on the U.S. market. The high fat content and small-scale processing contribute to a retail price that is at least twice as expensive as traditional sour cream. Nevertheless, sales are growing. Its increasing popularity is an indication of changing culinary habits promoted by growing population diversity and exposure to European culture. Although crème fraîche is far from being a mainstream product on the U.S. market, it is an interesting addition to the dairy case and can be found in many specialty stores.

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9

Yogurt and Sour Cream: Operational Procedures and Processing Equipment

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I. INTRODUCTION

Yogurt, also called *dadhi* in India, *leben* in Egypt, Iraq, and Lebanon, *tiaourti* in Greece, *madzoon* in Armenia, *yaourt* in Russia and Bulgaria, and *mast* in Iran, is one of the oldest and most popular forms of fermented milk in the world. It has been an important food in Asia, central Europe, and the Middle East, especially in countries bordering the east Mediterranean coast, since 5000 B.C. (1). One of several legends suggests that yogurt was first discovered after goat milk, stored in gourds in the hot climate of Mesopotamia, naturally formed curd (2). A brave soul tasted the curdled mass, reported it to be delicious and survived, thus yogurt-making soon thereafter became an art. Commercial production of yogurt reportedly began in Europe in the early twentieth century, after Nobel laureate Dr. Elie Metchnikoff published his endorsement of regularly consuming cultured milks, especially yogurt, for "prolongation of life" (3–5). Commercialization of yogurt in the United States reportedly began in 1939 in New York City (3).

Yogurt is an acid gel resulting from the fermentation of skim or low-fat milk by lactic acid bacteria; sour cream or cultured cream is an acid gel resulting from the growth and activity of lactic acid bacteria in light to heavy cream. It is likely that the production of sour cream was delayed only by the discovery of separation and utilization of cream, since the methodology of sour cream production does not differ profoundly from that of yogurt production.

Between 1980 and 1999, yogurt consumption typically rose (Table 1). The most significant growth (23.3%) occurred in 1983. Not shown in the table is the reported 10.5% increase in yogurt sales experienced between 1999 and 2000, with an additional increase of 7.9% through 2001 (6). Furthermore, sales of yogurt and yogurt drinks topped 2,326 million during the 52 weeks ending March 19, 2002, a 7.7% increase from the previous year (10). Although generally on the rise, consumption of yogurt in the United States, at 2.0 kg per capita, pales in comparison to international consumption patterns. Per capita consumption in Japan and France is about 3.6 and 20 kg, respectively (7). The disparity in numbers between the United States and other parts of the world shows the potential for growth in yogurt consumption. Mounting consumer awareness of the health benefits of yogurt and

Table 1 U.S. Yogurt Sales and Per Capita Sales, 1980–2000

Year	Sales (million kg)	Percent change (from previous yr)	Per capita sales (kg)	Percent change (from previous yr)
1980	258	3.6	1.1	0
1981	254	−1.8	1.1	−0.4
1982	272	7.1	1.2	8.3
1983	336	23.3	1.5	23.0
1984	381	13.5	1.6	12.5
1985	426	11.9	1.8	11.1
1986	454	6.4	1.9	5.0
1987	471	3.8	2.0	2.4
1988	494	5.0	2.0	4.7
1989	466	−5.7	1.9	−6.7
1990	452	−3.0	1.8	−4.8
1991	482	6.6	1.9	5.0
1992	490	1.7	1.9	0
1993	500	1.9	1.9	0
1994	552	10.3	2.1	9.5
1995	605	9.6	2.3	8.7
1996	582	−3.7	2.2	−4.0
1997	626	7.5	2.3	6.3
1998	620	−0.9	2.3	−2.0
1999	607	−2.0	2.2	−4.0

Source: Ref. 41.

the increasing varieties of yogurt products and flavors in the marketplace will contribute to continued growth in yogurt sales in the United States.

One innovation that has had a profound impact on the acceptability and increased sales of yogurt in the United States since fall 1998 is portable yogurt (8). Portable yogurt has been targeted to three different segments of the population: toddlers (Yumsters), children (Go-GURT, Trix yogurt, YoSqueeze), and adults (Exprèsse). Go-Gurt sales in 1999 were 63 million, with 24.8 million unit sales, up more than 500% over the previous year when the product was introduced (9). Sales of yogurt shakes and drinks (Danimals, Nouriche, Yo-J) were reported to increase 270% between 1999 and 2001, as the fairly new category gained strength (6). In 2001, kids were targeted with DANNON SPRINKL'INS, low-fat yogurt with special "surprise" toppings, and Kemps Spoonz'N Yogurt, with edible vanilla cookie spoons attached to 4-oz (140 g) cups of yogurt. Even toddlers are a target market for yogurt. Stonyfield Farm introduced Yo Baby organic whole milk yogurt, in convenient 4-oz cups, in the fall of 1999 (8). Although adults still account for 84% of the yogurt category volume, marketers understand the importance of establishing eating habits and brand loyalty in the young (10).

In fall 2001, DANNON introduced La Crème, a product with a creamy texture in five different subtle flavors, available in packs of four 4-oz cups (11). This product was created for people who like the concept of yogurt but don't necessarily like its sharp taste. In January 2002, Whips! a mousse-like whipped yogurt, was introduced to the market by Yoplait (11). Mild and sweet in flavor and fluffy in body, whipped yogurt may be more acceptable to individuals who have not embraced the sharp flavor of traditional yogurt, and it may significantly enhance sales in the yogurt category in 2002–2003.

U.S. sour cream sales constitute only about 28% of yogurt sales (13). In 1999, sour cream sales topped 560 million, or 425 million units, up 10% from the previous year (9). In 2000, sour cream sales topped 587 million, up 3.3% from the previous year, compared to 2.1 billion in sales for yogurt (12). In 2001 and 2002, dollar sales grew to over 655 and 669 million, up 10.2% and 9.7%, respectively (10,14). Sour cream, primarily used as an ingredient and potato or ethnic food topper, did not experience many innovations until recently, including convenient packaging, eye-catching graphics, and seasonings (14). One innovation that added life to the sour cream market is squeezable sour cream, first introduced by Kemps in 1999 (15). Since then, many companies have introduced squeezable varieties. Inclusion of chive and onion flavors to squeezable sour cream by Shamrock Farms improved volume sales in 2000 (13,14) The sour cream category also benefits from the growing popularity of Mexican foods among U.S. consumers (14).

II. PROCESSING EQUIPMENT AND MILK HANDLING PRIOR TO YOGURT AND SOUR CREAM PRODUCTION

The equipment needed for processing yogurt and sour cream is interchangeable, so both products may be manufactured at a single facility. Necessary components of the processes include holding tanks for raw ingredients and equipment for separation, standardization of the fat content, homogenization, heat treatment (pasteurization), culture preparation (if direct-set cultures are not used), and inoculation, packaging, and storage.

For production of Grade A products, milk suppliers and processors must comply with recommendations outlined in the Grade "A" Pasteurized Milk Ordinance (16). For instance, "All multi-use containers, equipment and utensils used in the handling, storage or transportation of milk shall be made of smooth, nonabsorbent, corrosion-resistant, nontoxic materials, and shall be so constructed as to be easily cleaned (16)." Farm holding/cooling tanks, welded sanitary piping, and transportation tanks shall comply with the applicable requirements of items 10p and 11p of section 7 in the PMO (16).

A. Holding Tanks

High-quality raw ingredients are essential to manufacture high-quality final products. Thus, holding of raw materials must meet legal standards outlined in the PMO (16). Grade A raw milk or pasteurized cream must be held at 4°C in approved silos or vertical tanks, for no longer than 72 hours prior to use in yogurt or sour cream production. Agitation in the silo tanks will prevent separation of cream by gravity. The agitation must be smooth and gentle. Accurate temperature indicators and temperature recording charts are legal requirements.

B. Clarification/Separation

If raw milk is the starting material in the yogurt or sour cream processing facility, separation is necessary because the starting materials must contain specified fat levels. Separation of milk into skim and cream fractions must be conducted in approved equipment.

Centrifugal separators not only separate the cream and skim fractions but remove solid impurities from milk. Milk is forced through discs with vertically aligned distribution holes at a specific distance from the edge of the disc stack (Fig. 1) (1). Under centrifugal force, milk fat globules and sediment (somatic cells, hair, etc.) are forced radially in the separation channels either outwards or inwards, based on their density relative to that of

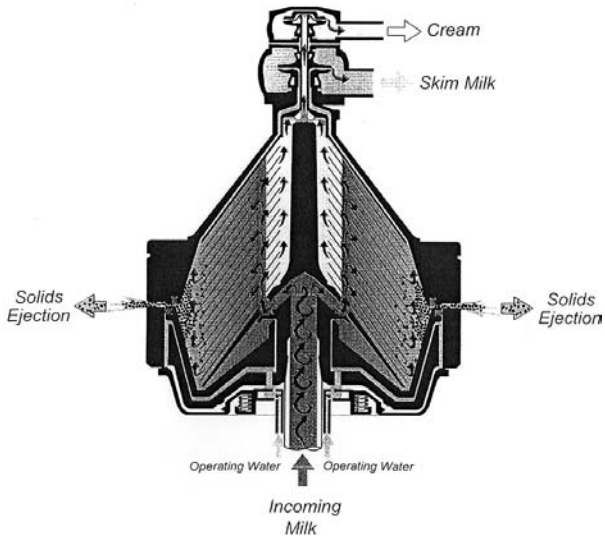

Figure 1 Cross-section of a centrifugal separator bowl with outlets for skim milk (lower) and cream (upper). Milk enters the disk stack at the bottom, through distribution holes. Solids ejection occurs by intermittent opening of the sedimentation space at the periphery of the bowl. (Courtesy of Tetra Pak.)

the continuous medium (skim milk). The skim milk portion moves outwards toward the boundary of the disc stack and through a channel between the top of the disc stack and the conical hood of the separator bowl to a concentric skim milk outlet. The cream moves inwards in the channels, toward the axis of rotation, to the cream axial outlet, above the skim outlet. Solid impurities are separated and thrown outward along the undersides of the discs to the boundary of the separator bowl and are collected in the sediment space. The solids that collect in the sediment space may be removed manually or automatically, depending on the type of separator. In self-cleaning or solids-ejection separator bowls, solids are ejected at 30- to 60-min intervals during separation, depending on the system. Skim and cream are channeled to receptacles for standardization and further processing (1).

C. Standardization

The fat content in cream may be controlled automatically during the separation process or via direct in-line standardization. During automatic separation, the volume of cream discharged from the separator is controlled by a throttling valve that is located at the cream outlet. Progressively larger amounts of cream, with a successively diminishing fat content, are discharged from the cream outlet when the valve is gradually opened. Changes in the cream discharge are matched by equal and opposite alterations in the skim milk discharge. In modern processing plants with diverse products, direct in-line standardization is usually combined with separation. Control valves, flow and density meters, and a computerized control loop are used to adjust the fat content of milk and cream to desired levels (1).

Most commonly, whole milk is preheated to 55–65°C in the regeneration section of the high-temperature short-time (HTST) pasteurizer prior to separation. Following separation, the cream is standardized to a preset fat level, and the fraction intended for standardization of milk is routed and remixed with the proper amount of skim milk to

attain the desired fat content. The surplus cream is directed to a separate cream pasteurizer, while the standardized milk flows through the pasteurizer. A typical processing line may be set up much like what is shown in Fig. 2.

Pressure must be strictly controlled in order to enable accurate standardization. This is achieved with a constant-pressure valve located just before the skim milk outlet. The cream-regulating system maintains constant fat content in the cream discharged from the separator by adjusting the outlet flow of cream, regardless of variations in the throughput or in the fat content of the incoming whole milk. A ratio controller mixes cream of constant fat content with skim milk in proportions that result in standardized milk of a specified target fat content (1).

D. Homogenization

Homogenization is a critical step in the process of making high-quality yogurt or sour cream. The milk fat globules in raw milk may be as large as 2.0–20.0 μm in diameter (17). At that size, and in their raw state, fat globules tend to rise to the surface and clump or coalesce. Homogenization is used to stabilize milk fat against gravity separation. The process of forcing fat globules through tiny holes under high pressure breaks the large globules into smaller ones, about 0.1–3 μm in diameter, rendering them less likely to coalesce and rise to the surface (18). Homogenization is most efficient when fat globules are in the liquid state; that is, the milk has been preheated. Thus, milk is processed through a heat exchanger in the regeneration section of the HTST (Fig. 3), where the temperature is raised to at least 60°C prior to homogenization (18).

There are two types of homogenizers: single-stage and two-stage. Both are composed of the same stainless steel parts, but a two-stage homogenizer has two sets of components. Homogenization takes place in the first stage of the homogenizer (10–25 MPa/100–250 bar/90–225 atm/1320–3300 psi). The second stage (48 bar/47 atm/696 psi) makes homogenization more efficient, by supplying controlled back-pressure. It also breaks apart any fat clumps that may have formed after the first stage. When liquid passes through the narrow gap of the homogenizer (Fig. 4), the flow velocity increases until the pressure is so low that the liquid starts to boil (1). When the liquid leaves the gap, the speed decreases and the pressure increases again. The liquid stops boiling and the steam bubbles implode. Theories suggest that fat globules may be broken down by either (a) numerous small

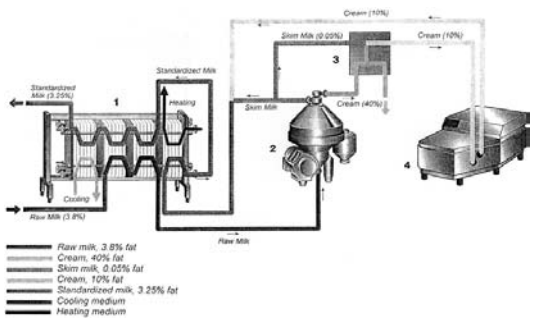

Figure 2 Product flow under partial stream homogenization (skim milk is not homogenized): 1. Heat exchanger; 2. centrifugal separator; 3. Automatic fat standardization device; 4. Homogenizer. (Courtesy of Tetra Pak.)

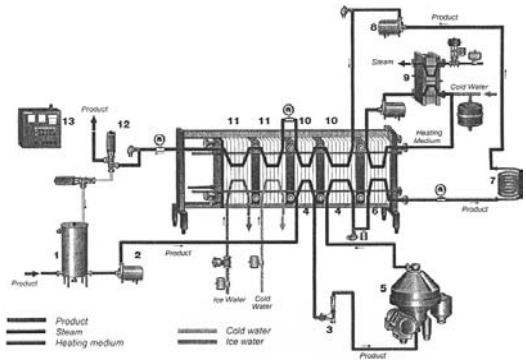

Figure 3 The complete pasteurizer consists of 1. balance tank; 2. feed pump; 3. flow controller; 4. regenerative preheating sections; 5. centrifugal clarifier/separator; 6. heating section; 7. holding tube; 8. booster pump; 9. hot water heating system; 10. regenerative cooling sections; 11. cooling sections; 12. flow diversion valve; 13. control panel. (Courtesy of Tetra Pak.)

eddies (or whirls) present in high-velocity flow systems or (b) cavitation, shock waves created when steam bubbles break and disrupt the fat globule (1).

E. Pasteurization/Heat Treatment

The only step in the dairy processing system in which inactivation of pathogenic microorganisms is guaranteed is pasteurization. For this reason, pasteurization is the most critical segment of the processing line. Pasteurization may be conducted in a vat batch system, also called low-temperature long-time (LTLT) or in a continuous high-temperature short-time (HTST) system. Of course, strict sanitation is critical up to and beyond pasteurization to assure the safety and quality of dairy products.

In a LTLT system, milk, yogurt mix, or sour cream mix is continuously agitated in a single tank, at a set temperature (legally at least 62.8°C) for a given time (legally at least 30 min if at 62.8°C) to guarantee inactivation of pathogens (16). In the case of HTST, milk must be held at 72°C for 15 sec to be legally pasteurized (16). For the production of yogurt

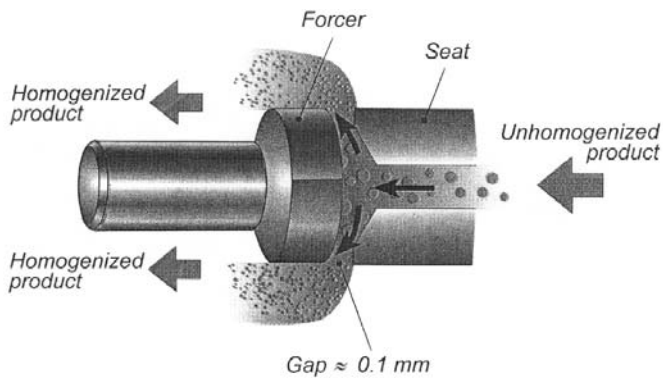

Figure 4 During homogenization milk is forced through a narrow gap where fat globules are shattered. (Courtesy of Tetra Pak.)

and sour cream, higher heat treatments are utilized in order to denature whey proteins, which enhances water retention and viscosity. Specific time-temperature combinations will be discussed in Secs. III and IV, independently.

There are several stages to the HTST pasteurization process:

1. Balance tank. Milk entering the HTST unit is collected in a balance tank, which contains a floater to prevent overfilling.
2. Regenerative preheating. Incoming milk is heated by outgoing milk (which is simultaneously cooled), separated by plates in the regeneration unit. Positive pressure is exerted on the side of pasteurized milk so pasteurized milk will not be contaminated if there is a system error.
3. Fat modification. Milk or mix is typically clarified/separated prior to homogenization, which occurs prior to final heating.
4. Heating section. Warmed milk enters the heating chamber, where it is heated by hot water/steam to 72°C (or higher).
5. Holding tube. Milk enters the holding tube, where it is held at a prespecified temperature and time (for instance, 72°C for 15 sec).
6. Flow diversion. If milk temperature drops below the target temperature (72°C), it is directed by a flow diversion valve back to the balance tank because every particle of milk must be maintained at 72°C for 15 sec for legal pasteurization. At startup, all milk is diverted to the balance tank until the target temperature is reached.
7. Regenerative cooling. After holding, milk enters the regeneration unit, where it is cooled by incoming milk (which is simultaneously heated as in #2).
8. Cooling. If fluid milk is the desired product, the milk is cooled to a final temperature of 4°C in a cooling section with ice water or glycol. If a mix is to be used in the production of sour cream, yogurt, or another product, the mix may be cooled to the appropriate inoculation temperature rather than to 4°C.

The HTST system has many checks and balances to assure product safety. Temperature charts are documentation and legal proof that adequate pasteurization temperature and time have been employed. The holding tube and flow diversion valve guarantee that every particle of milk is legally pasteurized. Higher pressure on the pasteurized product side than the raw product side and leak detectors prevent cross-contamination.

F. Culture Preparation

Culture quality is of utmost importance in the production of high-quality cultured dairy products. Culture manufacturers often work closely with processing facility operators to effectively meet specific needs. Culture manufacturers, through significant research efforts, develop unique culture combinations for a given product. For instance, all yogurt contains the thermophilic cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, but the specific strains of each are carefully combined in order to obtain particular flavor, body, and texture characteristics in yogurt. Specifically, some strains, such as the so-called ropy strains of *L. delbrueckii* subsp. *bulgaricus* or *S. thermophilus* that produce exopolysaccharides, may be selected for their ability to add viscosity to yogurt (19,20).

Several types of culture forms are available, including (a) liquid (for propagation of mother culture; rarely used today), (b) deep-frozen concentrated cultures (for propagation of bulk starter), (c) freeze-dried concentrated cultures in powder form (for propagation of bulk starter or DRI-VAC, for preparation of mother culture), and (d) deep frozen or freeze-dried, super-concentrated cultures in readily soluble form for direct inoculation of

the product (direct vat set or DVS). The use of frozen or freeze-dried cultures eliminates the need for small dairy plants to make cultures or operate a culture room (21). Larger plants are typically supplied with frozen or freeze-dried cultures for the manufacture of bulk starters in aseptic bulk culture rooms (21).

DVS cultures are so easy to use that they are commonly utilized today, but it is useful to describe the procedure for preparation of bulk starter. To begin, an understanding of the terminology is necessary. Commercial culture is the original culture obtained from the culture manufacturer. Mother culture is the culture prepared from the commercial culture, at the processing facility. An intermediate culture may be made for large volumes of bulk starter. Bulk starter is the starter used in production. The production of mother culture, intermediate culture and bulk starter are essentially the same. The steps include (a) heat treatment of the skim milk (or reconstituted skim milk with 9–12% dry matter) medium, (b) cooling to inoculation temperature, (c) inoculation, incubation, (d) cooling of the finished culture, and (e) storage of the culture (1). Heat treatment to 90–95°C for 30–45 min destroys microorganisms and bacteriophages, denatures proteins, and expels dissolved ozygen, thus improving the properties of the medium for culture propagation (1). Inoculation temperature and inoculum level are defined by culture manufacturers, but typically inoculation temperature ranges are 20–30°C for mesophilic bacteria and 42–45°C for thermophilic bacteria (1). Cooling and storage conditions and shelf lives of cultures vary. Generally, deep-frozen and freeze-dried cultures can be stored for at least 12 months at −18°C and −45°C, respectively (1). It is of utmost importance for consistency to be exercised in culture handling if a consistent product is desired.

A separate room in the dairy plant for preparation and propagation of starters is one important element in production of quality yogurt because it limits opportunities for contamination by airborne yeast, mold, and bacteriophages (1). Bacteriophages are essentially viruses that infect specific cultures and cause failure of lactic acid production. Each strain of culture has a different level of sensitivity to bacteriophage. Because it is impossible to entirely eliminate bacteriophage from a dairy plant operation, control measures must be employed. Aseptic techniques for propagation of starter cultures, sterilization of air and equipment, culture rotation of phage-unrelated, or use of phage-resistant strains is necessary to control phage (22).

Specific culture combinations, incubation temperature, and inoculation rates utilized in yogurt and sour cream production will be discussed in Secs. III and IV, independently.

III. YOGURT PROCESSING

A. Standard Yogurt Mix Elements and Processing Parameters

Yogurt may be classified as plain, extract- or concentrate-flavored (vanilla, lemon, etc.), sundae-style (fruit-on-the-bottom or fruit-on-top), Swiss-style (prestirred, preblended, or French), portable, drinkable, extra-creamy, whipped, or concentrated. Each yogurt style will be discussed separately in the following pages, but because yogurt mix processing is common to all yogurt-based products, yogurt mix elements and processing parameters will first be generalized in this section. Equipment considerations will be elaborated in a later section.

1. Standard Yogurt Ingredients

In the United States, standards of identity are legally defined in the Code of Federal Regulations (CFR) of the Food and Drug Administration (Table 2) (23). According to the

Table 2 Composition[a] Standards for Yogurt in the United States

	Yogurt (%)	Low fat yogurt (%)	Nonfat yogurt (%)
Fat	>3.25	>0.5 < 2.0	<0.5
Milk solids not fat	>8.25	>8.25	>8.25
Titratable acidity	>0.90	>0.90	>0.90

[a] Prior to addition of bulky flavors.
Source: From Ref. 23.

CFR, before the addition of bulky flavors, yogurt must contain not less than 3.25% milkfat and not less than 8.25% milk solids-not-fat; have a tritratable acidity of not less than 0.9%, expressed as lactic acid; and contain *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* cultures (23). Lowfat and nonfat yogurt must meet the same standards, except reduced fat content is defined as 0.5–2% fat and no more than 0.5% fat for lowfat and nonfat yogurts, respectively.

Milk chosen for yogurt manufacturing must be of high bacteriological quality. It must have low bacterial numbers (<100,000 cfu/mL) and be free of substances that impede the development of culture, such as antibiotics, bacteriophage, natural inhibitors in milk, or residues of cleaning or sanitizing solutions (22). A high-quality product cannot be produced from inferior ingredients. Production facilities test for the presence of antibiotics directly on site using highly sensitive detection methods. Milk with traces of antibiotic residues must be rejected since small quantities can affect the activity of the starter culture, and antibiotics are a public health hazard (22). Bacteriophages were described previously. Natural inhibitors in milk that may slow the starter cultures in yogurt include antibodies (agglutinins, lactenins), lactoperoxidase, thiocyanates, some acids (formic, acetic, butyric, and decanoic), and somatic cells (22). Sanitizing solutions must not be rinsed off equipment but must be allowed to naturally drain from equipment, or inhibition of cultures may result.

The fat and solids of milk are normally standardized so that the fat content is between 0 and 10%. A fat content of 0.5–3.5% is most common. Optional dairy ingredients that may be used to produce yogurt include cream, milk, partially skimmed milk, or skim milk, used alone or in combination (23). Concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, or whey modified by partial or complete removal of lactose and/or minerals may be used to increase the MSNF content (23).

Although yogurt can be made without stabilizers, whey separation, or syneresis, is likely; product appearance suffers when stabilizers are not used. Stabilizers commonly used in yogurt, along with their functional properties and usage levels, are listed in Table 3. Blending gums, starch, emulsifiers, and in some cases corn syrup solids supplies emulsification and stabilization to yogurt, promoting desirable body and texture (24). Combining starch and xanthan gum provides a creamy texture to low-fat yogurt; carrageenan and pectin work well together at the low pH found in yogurt; agar and gelatin help bind water and prevent syneresis (24).

An increase in the total dry mater content, particularly the proportion of casein and whey proteins, will result in a firmer yogurt coagulum, and the tendency for syneresis will be reduced (25). Evaporating the milk, adding nonfat dry milk (NFDM), or partial ultrafiltration increase yogurt curd firmness (25). Most yogurt is fortified with 2–4% NFDM to improve stabilization and consistency. A level of total solids in the range of 14–18% is recommended; total solids in excess of 25% hinder starter activity (22).

Table 3 Common Stabilizer Usage in Yogurt Products

Stabilizer	Function	Recommended usage level
Agar	Carbohydrate derived from certain species of red algae. Provides viscosity.	0.25–0.7%
Carboxy-methyl cellulose	Carbohydrate. Thickens, adds viscosity, reduces syneresis.	0.2–1.5%
Carrageenans (*kappa-, iota-, and lambda-*)	Carbohydrates derived from red seaweed. Provide viscosity and gelation properties. Stable at pH 3.5–4.0. *Kappa-* produces strong, rigid gels. *Iota-* gels are weaker but resist syneresis. *Lambda-* interacts strongly with proteins.	0.2–1.5%
Gelatin	Protein derived from animal collagen. Gelation properties, prevents syneresis.	0.3–2.0%
Guar gum	Carbohydrate derived from guar seeds. Thickening properties. Stable over a wide pH range, but not at high temperatures.	0.2–1.5%
Locust bean gum	Carbohydrate derived from carob seeds. Thickens, contributes to gel properties, reduces syneresis.	0.2–1.5%
Maltodextrin	Carbohydrate. Provides a soft, custard-like reversible gel that mimics the texture and gel character of gelatin and hydrocolloid gums.	1–5%
Modified food starch (corn, potato, or tapioca)	Carbohydrate. Provides thickness, gelation properties.	0.1–0.7%
Pectin (low methoxy [LM] for yogurt, high methoxy [HM] for drinkable)	Carbohydrate derived from plant material, commonly citrus or apple. LM requires calcium to gel, but not high solids. Provides viscosity, gelation properties.	0.08–0.12% 0.08–0.20%
Whey protein (whey protein concentrate, whey protein isolate)	Protein derived from cheesemaking process. Whey proteins are available in many forms. Function like emulsifier/stabilizer. Provide water binding and gelation properties.	1–2%
Xanthan gum	Carbohydrate derived from bacterial fermentation. Provides high viscosity, sometimes slimy or stringy.	0.2–1.5%

Source: Refs 24, 26, and 42.

According to the CFR, a multitude of nutritive carbohydrate sweeteners may be used as ingredients in yogurt, including sugar (sucrose), beet or cane; invert sugar (in paste or syrup form); brown sugar, refiner's syrup; molasses (other than blackstrap); high fructose corn syrup; fructose; fructose syrup; maltors, maltose syrup, dried maltose syrup; malt extract, dried malt extract; malt syrup, dried malt syrup; honey; maple sugar; or any of the sweeteners listed in Part 168 of the CFR, except table syrup (23). It should be noted that sweetener levels greater than 12% can be inhibitory to cultures. Alternatively, nonnutritive sweeteners, such as Aspartame or Nutrasweet may be utilized to minimize caloric or cariogenic properties associated with nutritive carbohydrate sweeteners. Additionally, FDA-approved flavoring ingredients, color additives, and stabilizers are allowed. Vitamin A and D addition, which is optional, is specified in the CFR (23).

In Table 4, a typical yogurt formulation with functional properties of common yogurt ingredients is shown. All yogurt ingredients, except culture and inclusions, must be blended completely in order to allow full hydration of all ingredients prior to pasteurization and homogenization.

2. Standard Yogurt Processing

An overall scheme of yogurt processing is included in Fig. 5. Milk or standardized yogurt mixes are heat-treated to high temperatures well above minimum legal pasteurization. LTLT pasteurization at 85°C for 30 min or 88°C for 15 min or HTST pasteurization at 88–91°C for 30 sec to 5 min are typically employed. The purpose is twofold. First, high temperatures destroy indigenous bacteria, particularly themodurics, which reduces competition and enables rapid growth of the culture bacteria. Second, high temperatures denature about 70–80% of the whey proteins. The denaturation not only improves culture nutrient utilization but also increases water-holding capacity and curd strength, minimizing syneresis.

Yogurt mix may be homogenized after preheating in an HTST system, or at the end of the LTLT or HTST pasteurization process. Although unhomogenized milk may be used for yogurt, most manufacturers homogenize yogurt mix in order to prevent creaming during the incubation period, to assure uniform distribution of the milk fat, and to stabilize the coagulum against whey separation (26). Further, homogenization, followed by high temperatures, improves yogurt viscosity. Homogenization may be single-stage at 1800–2500 psi or two-stage at 2000/500–1500/500 psi. The air content of milk used to make yogurt should be minimized to maximize homogenization efficiency and minimize fouling during heat treatment. Air is typically added when MSNF content is modified by addition of milk powder. In such cases, the milk should be deaerated prior to yogurt making.

As the homogenized yogurt mix containing stabilizers and/or sweeteners is cooled to 38–43°C, a 2–5% inoculum of an appropriate liquid culture is added. When concentrated

Table 4 A Standard Yogurt Formulation, Including Ingredient Functionality

Ingredient	Component (%)	Functionality
Skim milk	42.9	Body and viscosity, smooth mouthfeel, prevents syneresis by stabilizing protein gel
Whole milk	29.6	Provides protein gel, body and viscosity, smooth mouthfeel
Fruit puree	15.0	Color and flavor
Sucrose	5.5	Sweetness, increases total solids
Nonfat dry milk	3.4	Firmness and consistency of protein gel, water binding (improves body and prevents syneresis)
Whey protein concentrate	2.4	Firmness and consistency of protein gel, water binding (improves body and prevents syneresis)
Carrageenan	1.0	Consistency and viscosity of protein gel, water binding (prevents syneresis), maintains gel structure after cooling
Agar	0.2	Consistency and viscosity of protein gel, water binding (prevents syneresis), maintains gel structure after cooling
Yogurt cultures	0.01	Produce acid to form protein gel and acetaldehyde to provide yogurt flavor

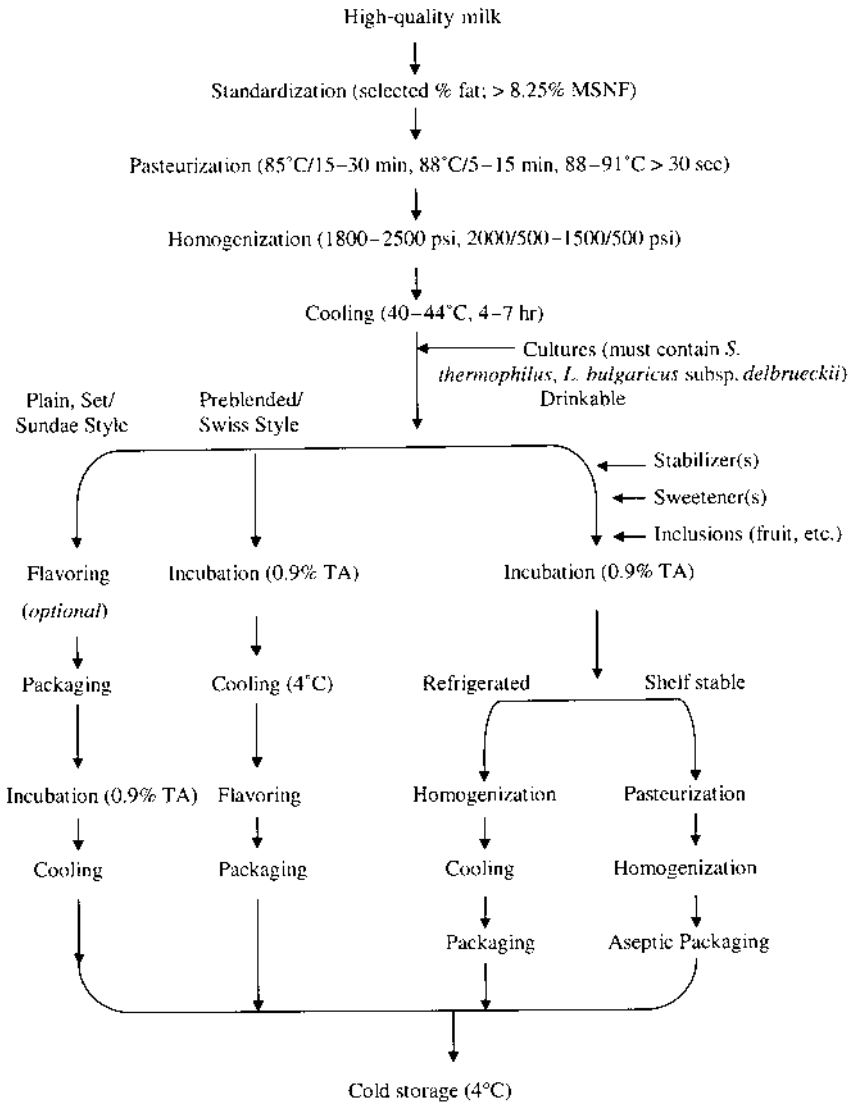

Figure 5 Manufacture of yogurt-based products.

DVS culture is added, this percentage is decreased. Inoculum level and incubation temperature will depend on the culture manufacturer's recommendations and final product characteristics. The cultures required for yogurt production are *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in nearly a 1:1 ratio at inoculation. Yogurt fermentation typically proceeds optimally between 40 and 43°C. Temperatures of 35–40°C favor *S. thermophilus* (acid production) whereas 43–45°C favors *L. bulgaricus* (acetaldehyde production). The growth of cocci and rods, and their ratio, change in yogurt during incubation. At first, the streptococci grow faster due to the formation of growth factors by the rods (25). As the acid level in yogurt increases, the cocci are slowed down and the rods grow faster because of the growth factors produced by the cocci (25).

Live yogurt cultures are present throughout processing and consumption. Although most strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* do not survive in the human intestinal tract, other microorganisms not only survive but also adhere to and proliferate in the human intestinal tract (27). Cultures typically utilized in yogurt because of their probiotic effects include *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus casei*, and *Bifidobacterium* subsp. Probiotics is a term used to describe microorganisms that, if consumed, exert beneficial effects upon health. Yogurt is an ideal food system for delivering probiotics to humans because yogurt buffers the stomach and raises its pH; yogurt's protein content and semisolid consistency give probiotic bacteria a chance to survive the stomach and adhere to the intestine (27).

Probiotic microorganisms must (5,27):

1. be nonpathogenic and nontoxigenic
2. adhere to the host's gastrointestinal tract and colonize it
3. be able to utilize nutrients and substrates available in the host's typical diet
4. survive passage through the host's digestive system
5. have good viability in the form delivered to the host
6. exert beneficial effects on the host by preventing infections or diseases, improving health, or improving nutrition

For the "Live and Active Cultures" logo from the National Yogurt Association to appear on a yogurt label, 10^8 cfu/g of lactic acid bacteria must be present at the time of manufacture (27). The lactic acid bacteria population may be 10^8 cfu/g at the time of production, but viability declines during storage. The therapeutic dose of probiotic bacteria per day is suggested to be 10^8 to 10^9 cells, so it is essential that products sold contain a minimum of 10^6 cfu/mL at the expiration date on the package (27). The viability of probiotic bacteria in yogurt depends on many factors, including (a) the strains used, (b) interaction among species present, (c) chemical/nutrient composition of the yogurt base, (d) final acidity, (e) presence of inhibitors, (f) dissolved oxygen, (g) inoculation level, (h) incubation temperature, (i) fermentation time, and (j) storage temperature (25). For instance, *Bifidobacterium* subsp. are susceptible to acids and oxygen, so their counts, in particular, may decrease significantly during storage (25). However, because *S. thermophilus* acts as an oxygen scavenger and creates an anaerobic environment, its presence can enhance the survival and growth of *Bifidobacterium* subsp. (27).

The inoculated warm mixture may be incubated in a vat or pumped into consumer containers, standard milk cans, or stainless steel vats as large as 1500 gal. The mix is then held at the optimum temperature for 3–7 hr, until a pH of 4.4 or a titratable acidity (TA) of 0.9–1.2%, as lactic acid, is attained. Optimum TA prior to chilling is 0.9 and optimum pH is 4.5. At this point, yogurt is either cooled or "broken" rapidly in order to halt culture activity. Optionally, appropriate flavorings may be added according to the desired end product.

Cup-set yogurt is more prone to wheying off than vat-set yogurt, so particular care must be taken to minimize handling of the warm cups of yogurt (28). Positive displacement pumps are least destructive to yogurt (22). Once yogurt containers are packed, they are transferred to a blast cooler until the temperature is lowered to about 5–7°C. The product can be held in a regular cooler until sale. Boxes of yogurt are palletized in circulating air chillrooms to maximize cooling rate (40).

Yogurt should be a smooth, viscous gel with a characteristic sour, green-apple flavor. Flavor compounds produced during fermentation by yogurt bacteria include lactic acid (sour), acetaldehyde (green apple), acetic acid (vinegar), diacetyl (buttery), and volatile fatty acids (butyric, caproic, capryllic, capric). Incubation temperature, inoculum level,

incubation period, choice or source of the culture (strains used), heat treatment of yogurt milk base, and pH of the finished product all influence the body, texture, and flavor of the final product. Other factors that affect yogurt quality include choice of milk, milk standardization, additives, deaeration, homogenization, culture preparation, incubation temperature, and plant design (1).

B. Plain, Extract- or Concentrate-Flavored, Sundae Style

Simply put, set yogurt is incubated and cooled in the retail package. Set yogurt may be plain or may include color and/or flavoring and/or fruit. Plain or "natural style" yogurt has no added flavoring material or fruit and is seldom, if ever, sweetened. Plain yogurt has a tart or sour, somewhat gree apple-like flavor, due to the high levels of lactic acid (0.9–1.2%) and acetaldehyde (5–40 ppm) present, respectively (3). Sweeteners, fruits, and/or flavorings tend to mask some of the naturally occurring acid and acetaldehyde flavor compounds in flavored yogurts.

If fruit is incorporated into the bottom of the cup, the yogurt is called sundae style or fruit-on-the-bottom. Fruit is rarely added to the top of the yogurt (fruit-on-top) in the United States. Ingredients used in Western-type sundae-style yogurt include coloring agent(s), flavor extract(s) or concentrate(s), and/or sweeteners, which are added to the yogurt base (milk base is colored according to the given flavor). Eastern-type sundae-style yogurt contains no coloring, flavoring, or sweetener in the yogurt base (milk base is white). Fruit-on-top style yogurt is made by filling yogurt cups with milk base first, followed by the addition of flavoring material on top.

The amount of fruit flavoring, fruit preserves, puree, or jam varies from 1.25 to 2.0 oz (37–59 g) per 8 oz (230 g) cup. In addition to the lactic acid and acetaldehyde flavors being somewhat masked by sweeteners and flavorings, production of these flavor compounds is limited because the metabolism of these end products by *L. bulgaricus* and *S. thermophilus* is inhibited by high sugar concentrations (3).

C. Swiss-Style Yogurt

The most popular type of yogurt in the United States is stirred. Stirred yogurt may also be referred to as Swiss-style, French, prestirred, or preblended yogurt. Although the yogurt base used to manufacture plain, Swiss, and sundae-style yogurts may be essentially the same, the manufacturing processes differ. Stirred yogurt is typically incubated in tanks and cooled before flavor addition and packaging. However, some facilities blend the fruit and flavors prior to incubation and allow the yogurt to set in retail containers.

Due to the differences in processing methods, set and stirred yogurts have markedly different textures (25). Sundae-style yogurt is allowed to set in the cup without being disturbed, but stirred yogurt structure is broken upon mixing with fruit. Thus, it is important to formulate Swiss-style yogurt appropriately to obtain the desired texture after blending. The texture of yogurt can be manipulated by formulation and processing techniques. Some of the factors that influence firmness in yogurt include casein or fat content of the milk, homogenization, heat treatment, yogurt cultures, pH, and incubation temperature (25). Casein enhances curd firmness, but as fat level increases, gel firmness decreases because the fat globules interrupt the protein network (25). Homogenization and heat treatment of the milk enhance firmness (25). Because yogurt structure is broken during the stirring process, exopolysaccharide-producing strains of *L. bulgaricus* or *S. thermophilus* are particularly useful for Swiss-style yogurt because they provide viscosity (19,20). Yo-

gurt firmness increases with decreasing pH, but yogurt pH should not be less than 4.1 (25). At low incubation temperatures, it will take longer for a certain pH to be attained, but the finished product is much firmer (25).

D. Drinkable Yogurt

Although one way to manufacture drinkable yogurt is shown in the process diagram, Fig. 5, several options exist. Yogurt beverages are similar to stirred-style yogurt, but the coagulum is "broken" into a liquid form before being either cooled, repasteurized, or UHT treated, depending on the shelf life desired, and homogenized prior to packaging. If repasteurized or UHT-treated, bacteria are killed, so "contains live culture" will not appear on the label. At Dannon Co. (West Jordan, UT), Danimals Drinkables bottles are sent to an unscrambler and blown by air to conveyer belts connected to fillers (29). At the filler, the bottles are mechanically turned upside-down, rinsed, sterilized, and sent through to the filler. Bottles are sent to a 30-unit rotary wheel to be filled, then are capped with an aluminum foil seal. Every 15 mins, a random sample is tested for viscosity, pH, and color. Bottles are code-dated, then shrink-wraped in groups of four. Case packers group four-packs into sets of six, then the groups are corrugated, the boxes are code-dated and sent to the cooling room (29).

E. Extra-Creamy Yogurt

Four-ounce Dannon La Crème yogurts boast "a touch of cream" that provides "naturally smooth dairy taste and a rich, creamy texture that goes beyond traditional yogurts" (30). At approximately 4% fat, creamy yogurts contain 25% more fat than standard yogurts (approximately 3% fat) in the market. Modified food starch, corn starch, and Kosher gelatin help maintain the creamy texture. Since these products require only a formulation modification, processing need not be modified. However, because the creamy product may be packaged in smaller containers, the packaging line must be modified to accommodate the change in volume.

F. Portable Yogurt

Portable yogurt has met a consumer need for healthful portable foods. Go-GURT, Exprèsse, YoSqueeze, and Yo-Stix portable yogurts, in 2.0- to 2.5-ounce single-serve tubes, may be frozen, then packed in a lunch box to be eaten later in the day (13). It is likely that higher levels of stabilizers are used in these portable yogurts than standard yogurts in order to maintain desirable viscosity even after freezing. Although the manufacturing process is similar to that of standard yogurt, significant modifications must be made to the filling and packaging lines to accommodate filling and sealing of the convenient tubes.

G. Frozen Yogurt

Frozen yogurt, which can be soft or hard, resembles ice cream in physical structure more than it does yogurt. Higher levels of sugar (11–15%) and stabilizers (about 0.85%) than used in yogurt are required for frozen yogurt to maintain air bubble structure during freezing (1,22). Incubation time is extended due to the higher level of carbohydrates (1). No federal standards apply to frozen yogurt, but several states have regulations requiring a minimum titratable acidity. Yogurt for frozen yogurt is typically high in milk solids (25%) and incubated to high titratable acidities (1.50–2.00). A small amount of yogurt (10–15%)

is then blended with an ice cream mix and frozen like ice cream. An alternative method is to ferment a large amount of the base (30-100%) to a low titratable acidity (0.30–0.90) prior to blending and freezing (28). Ice cream and frozen yogurt are frozen in a scraped-surface heat exchanger. As sensible heat and latent heat are removed from the product in the freezer chamber, ice crystals form on the edges of the freezer barrel. Dashers scrape the frozen product from the freezer barrel and air cells simultaneously form within the serum, thus increasing product volume. The semi-frozen mass may be extracted from the freezer barrel at about $-8°C$ and serve "soft-serve" or filled into retail cartons and hardened in a blast freezer at about $-25°C$ (1).

H. Whipped Yogurt

Yoplait Whips! were the first whipped yogurt to enter the market. Light and fluffy in texture, Whips! contain 25% air compared to traditional yogurt: the same packages used for 6 oz of the standard product contain 4 oz of the whipped product. Air is whipped into the yogurt base using a proprietary process, much like the ice cream process but without freezing. Higher levels of sugar and stabilizers are used in whipped yogurt than used in standard yogurts to maintain air bubble structure during processing. Essential ingredients specific to whipped yogurts include gelatin, for foam structure; an emulsifier, for interphase stability; and pectin/starch, for viscosity and mouthfeel (31). During processing, the viscosity and temperature of the mix, as well as the flow rate, overrun, aging, and cooling kinetics are critical parameters that must be controlled for proper aeration of whipped yogurt (31).

I. Concentrated Yogurt

Concentrated yogurt, sometimes called strained yogurt or Labneh, is formulated much the same way as plain yogurt, described previously. The yogurt is incubated in tanks, concentrated by straining to about 24% total solids, and cooled before being packaged (22). Concentrated yogurt is used in recipes by the baking industry.

J. Other Yogurt Product Considerations

1. All Natural

"Natural" is an arbitrary term in the food industry because the FDA has not established a formal definition for the term, but the word means a lot to many consumers (32). Yogurt can be made successfully with a minimum of ingredients, when made under controlled conditions of processing and handling. All natural yogurt products are easily attainable. Suppliers must be consulted to obtain natural ingredients. If products are to be handled roughly, gums and stabilizers are almost a necessity, due to the fragile nature of the yogurt body.

2. Organic

Natural is not legally defined, but organic is. Ingredients used in organic yogurt must be certified organic. This means the products were obtained from certified organic farmland, feeds for cows were grown on certified organic farmland, and/or cows were raised on certified organic farmland. Certified organic farmland must be free of synthetic pesticides, herbicides, and fungicides for at least 3 years prior to being used to grow organic crops intended as ingredients or dairy cow feed (33). As of October 21, 2002, producers and

handlers must meet the National Organic Program's standards and be certified by a USDA-accredited certifying agent to sell, label or represent products as organic (34). Products labeled "100 percent organic" are products exclusively produced using organic methods. To be labeled "organic," at least 95% of the ingredients (by weight, excluding water and salt) must be organically produced (34). The remaining components can only be allowed natural or synthetic ingredients as stated in the Code of Federal Regulations (34). "Made with organic" products must contain 70–95% organic ingredients (34). Products with less than 70% organic ingredients can list the individual organic ingredients on the label (34).

3. Kosher

Several different symbols may appear on dairy products to indicate that the product is suitable for consumption by individuals observing dietary guidelines according to the Jewish faith. To receive Kosher certification, guidelines for handling of raw materials, processing, packaging, and distribution of foods and use of processing equipment must be followed (2). If a Kosher food is to be made after a non-Kosher food has been made, all equipment must be thoroughly cleaned, left idle for 24 hours, then washed with hot water prior to use for making Kosher foods (2). A rabbi or a Kosher Supervisory Agency must review and approve product processing procedures before Kosher symbols may be used. Products certified by the Orthodox Union are marked with the OU symbol (U enclosed by an O), placing a D beside the OU to signify the presence of dairy ingredients. Other Kosher symbols include CRC (Chicago Rabbinical Council), K, U, and parve or K-D (2). For sour cream or yogurt to receive Kosher certification, all ingredients used must be certified Kosher, including Kosher gelatin, colorings, and flavorings (2).

4. Multi-Packs

Four- and six-ounce size yogurts are options for consumers looking for yogurt snack options (33). These sizes are well-suited for multi-packs. Multi-packs of a single flavor or multiple flavors of cup or drinkable yogurt exist in the marketplace. Modifications must be made to the filling and packaging lines to accommodate the multi-pack feature.

5. Package Inclusions

Yogurt containers topped with separate enclosures of granola, candy, or other creative inclusions may also be found in the marketplace. Dannon Double Delights combine lowfat yogurt with dessert-like toppings in a dual-compartment container. Modifications must be made to the packaging line to accommodate the cup-topping or dual compartment features.

6. Yogurt with Vegetables

The natural sweetness of some vegetable purees (carrot, sweet potato, and Hubbard squash) lend themselves to addition in yogurt in place of fruits. In 2002, students from Washington State University demonstrated that the market may be ready for YoVè, an all-natural blend of yogurt and vegetables (12). Their product, a stirred yogurt with vegetable purees, was highlighted as a finalist at the 2002 Annual Meeting of the Institute of Food Technologists Student Association Product Development Competition, but it has yet to be launched in the marketplace. Minimal modifications to product formulation and processing line need to be made to produce yogurt with vegetables. Although the three flavors of YoVè were desirable to consumers upon tasting, the team determined that significant marketing and promotion would be necessary to overcome consumer perceptions that vegetables do not belong in yogurt and to guarantee the success of such a product.

Table 5 Troubleshooting Guide for Yogurt Production

Observation	Explanation	Solution
Bitter flavor	A bitter aftertaste may be due to culture contamination, excessive use of certain stabilizers, or poor quality ingredients.	Careful selection of ingredients and maintenance of culture quality is essential for high-quality yogurt.
Cooked flavor	A nutty or sulfur-like note may be apparent due to an excessively high heat treatment.	Careful control of the pasteurization process and appropriate balance of flavor systems can minimize or mask moderate cooked flavors.
High acetaldehyde flavor	Excessive "green apple" flavor indicates *L. bulgaricus*, stimulated at higher incubation temperatures (112°F), dominated the fermentation.	Maintain incubation temperature at or below 105°F to limit *L. bulgaricus* (*S. thermophilus* dominates below 105°F).
High acid flavor	Excessive lactic acid may cause extreme tartness (some sourness is appropriate) due to 1) extended incubation period, 2) elevated incubation temperature, 3) imbalance of bacteria, and 4) inadequate cooling to arrest culture activity.	Incubate yogurt between 41 and 43°C (105–110°F) for 4–7 hr or until the yogurt pH is between 4.45 and 4.65 and TA reaches 0.9%.
Low acid flavor	Under-incubation of the yogurt, imbalanced yogurt cultures, or partial failure (due to inhibitors) are possible causes for the lack of typical refreshing flavor and moderate tartness associated with good yogurt.	The pH should be <4.5 for appropriate acid flavor. Use appropriate heat treatment conditions to maximize culture activity. Reduce sweeteners in yogurt base (<12%).
Old ingredient flavor	When yogurt contains an old ingredient (NFDM, fruits, etc.), a "state" or bitter aftertaste persists after swallowing.	Careful selection and monitoring of ingredients is essential for high quality yogurt.
Oxidized flavor	A cardboardy, metallic, or tallowy off-flavor may result if oxidized ingredients are used in the yogurt base.	Careful selection and monitoring of ingredients is essential for high quality yogurt.
Rancid flavor	If lipase is not inactivated prior to ingredient blending, the enzyme may act on the milk fat.	Careful monitoring of pasteurization and homogenization temperatures is essential.
Gel-like body	Excessive product firmness, slickness, or rigidity due to overstabilization.	Use appropriate levels of stabilizer.
Grainy texture	Product lacks smoothness and uniformity due to undissolved solids, resulting from 1) unstable casein, 2) too-high homogenization temperature, 3) too-rapid acid development, 4) too-high incubation temperature, 5) excessive amount of culture, 6) incorrect stabilization system, 7) improper blending of yogurt base with fruit.	Allow complete hydration of milk powder and stabilizer(s), homogenize and incubate at appropriate temperatures, select stabilizer(s) carefully, blend yogurt base and fruit flavoring adequately.
Ropy body	Yogurt strings out when spooned due to certain bacterial polysaccharides or stabilizers.	A certain level of ropiness may be desirable. Balance culture and stabilizer(s).
Too firm body	Product resists mastication and gives a pudding-like sensation in the mouth due to overstabilization.	Use appropriate levels of stabilizer.

Table 5 Continued

Observation	Explanation	Solution
Weak body	Weak yogurt does not stand up in the spoon, and/or may appear watery due to 1) under-stabilization, 2) low casein or low total milk solids, 3) under incubation, or 4) low pasteurization temperature.	Careful yogurt base formulation and strict control of pasteurization temperature.
Color leaching	Pigment trails or leaching of color from fruit into yogurt, caused by low pH (<3.8) or incomplete blending of yogurt base with fruit flavoring material.	Use acid-stable colors within the typical pH range of yogurt (3.8–4.3). Properly blend yogurt base with flavoring material before filling.
Free whey	Whey release from curd may be due to (1) low milk solids, (2) excess acid development, (3) agitation during incubation, (4) insufficient milk pasteurization temperature or holding time, (5) poor product formulation, (6) inadequate stabilizer(s), (7) temperature fluctuations.	MSNF must be at least 8.25%. Practice good overall process control, (1) accurately monitor pH, (2) halt acid production between pH 4.5 and 4.0, (3) carefully select stabilizers, (4) minimize handling of warm yogurt.
Shrunken	Curd pulls away from carton due to tightening of curd structure; often associated with free whey.	Practice process control measures used to prevent free whey defect.

Source: Refs. 3 and 28.

K. Troubleshooting

Although the importance of quality ingredients, aseptic culturing techniques, and strict time-temperature controls has been highlighted, sometimes product quality suffers. Some of the defects that may be found in yogurt products, along with how to overcome the defects, are outlined in Table 5.

IV. CULTURED OR SOUR CREAM PROCESSING

A. Processing

1. Sour cream Ingredients

According to the CFR (Title 21, Section 131.160), sour cream must contain at least 18% milk fat and have a titratable acidity of at least 0.5%, expressed as lactic acid (36,37). Optional ingredients may be used to improve texture, prevent syneresis, improve flavor, or extend shelf life, but sour cream may not contain more than 0.1% sodium citrate, salt, rennet, nutritive sweeteners, flavoring, or coloring (36,37).

Production of high-quality sour cream (Fig. 6) must begin with high-quality milk and/or cream. If high viscosity is desired, or if mechanical agitation will be employed, the body may be improved by including stabilizers (hydrophilic colloids bind water), WPC, NFDM, or by using cultures that naturally excrete polysaccharides. Sour cream made with 18% fat may not need stabilizer (38). Stabilizers may be used at about 0.5%, and 0.2% citric acid may be used for flavor and enhanced culture activity. Occasionally, particularly outside the United States, chymosin, which is used in cheesemaking to augment coagu-

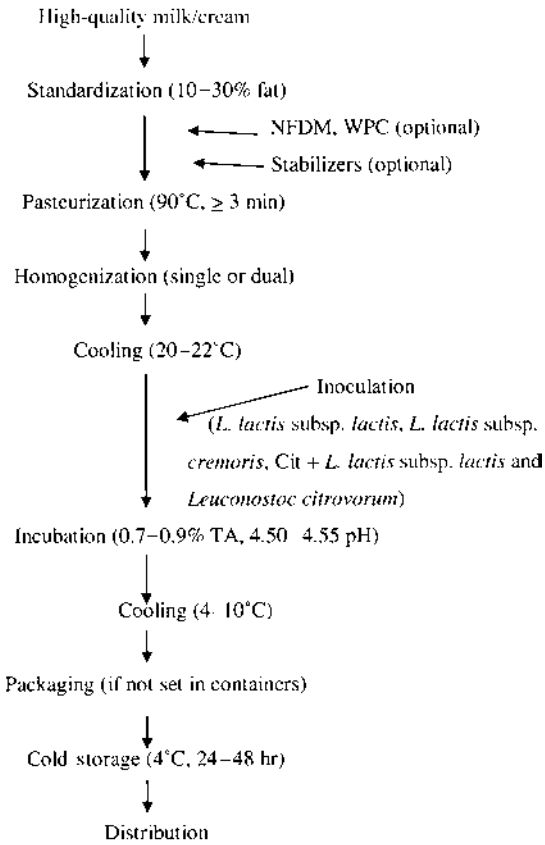

Figure 6 Production of sour cream.

lation, is used in sour cream production (0.5 mL single-strength chymosin/38 L cream) to improve gel strength (40).

Sour cream should have a mild, subtle, aromatic, and slightly acidic flavor (1,2) composed of lactic, acetic, butanoic and carbonic acids, and diacetyl (38). Desirable sour cream is bright, has a uniform smooth structure, and is relatively viscous or firm. Excessive addition of stabilizers and/or emulsifiers may impart an overly heavy body and can detract from the delicate, desirable flavor of cultured sour cream. Masking of flavor may occur when stabilizer/emulsifier levels exceed a recommended product usage level of 0.30–0.40% (w/w) (3). Under extended storage, quality breaks down as bacterial proteolytic enzymes cleave proteins into bitter peptides (1,2). Additionally, carbon dioxide and other aromatic substances can diffuse through the packages, causing off-flavor formation (1). Yeasts and molds can develop on the surface of sour cream readily if packages are not airtight. Sometimes sorbate is added to suppress the growth of yeast and mold, but since sorbate can also slow down cultures, the manufacturing procedure may need to be extended 3–4 hr (39).

Lowfat and nonfat sour cream are also available in the market. Because reduced-fat sour creams are inherently thinner than their full-fat counterparts, a combination of agar, guar gum, and carrageenan is a good mix for lower-fat versions of sour cream (38). Balance is important because excessive use of gelatin may yield a body much like Jello, and high levels of modified food starch may result in a pasty product body (38). Additionally,

modified food starch and/or sugar may be used to cut the harsh flavor common to reduced fat sour cream.

2. Sour Cream Processing

Typically, cream with 18–20% fat and 25–28% total milk solids is heated to 60–71°C in order to inactivate lipase prior to homogenization (Fig. 6). The cream may be preheated or completely pasteurized (either at 74–82°C for 30 min or HTST pasteurized at 85°C for 25–60 sec or 86–88°C for 3–5 min) prior to homogenization, depending on the processing plant layout. Preheating is necessary because homogenization of raw milk will result in rancid off-flavors in sour cream. The high pasteurization temperature not only inactivates bacteria, yeasts, and molds but denatures whey proteins, which improves water-binding capacity, so the sour cream is less likely to whey off. It has been noted (38) that LTLT pasteurization produces a strong-bodied sour cream compared to HTST pasteurization.

Homogenization is done at temperatures ranging from 40°C to 85°C at a pressure of 2,000–3,000 psi (14–21 MPa, 140–210 bar) (3). Cream with low fat (10–18%) is homogenized at 2,100–2,500 psi (15–20 MPa, 150–200 bar). However, cream with high fat (20–30%) is homogenized at 1,500–2,000 psi (10–12 MPa, 100–120 bar) because there is not enough casein to form membranes on the enlarged total surface area of the fat globules (1). Homogenization at higher temperatures has been shown to improve sour cream consistency (1). Additionally, researchers at Cornell University found that dual-homogenization (homogenizing twice) of highly heated light cream can improve sour cream smoothness and viscosity (2). A high degree of viscosity results from dual-homogenization due to extensive clumping of fat globules.

After pasteurization and homogenization, cream is cooled to 21–30°C and inoculated with 0.5%–2% starter culture. The starter organisms utilized to make sour cream include *Lactoococcus lactis* subsp *lactis* and *cremoris*. Citrate + *Lactococcus lactis* subsp *lactis* and *Leuconostoc citrovorum* are also used for aroma (1). *Lactococcus* can produce acetaldehyde while *Leuconostoc* can use acetaldehyde, so combining the two is useful, because excessive acetaldehyde is inappropriate in sour cream. Incubation can take place in a tank or in the packages. Either way, inoculated milk is held for 16–20 hr at 20–30°C (most typically 20–22°C) or until the proper acidity (0.70–0.90) and pH (4.50–4.55) are attained (1). After coagulation, the curd is quickly cooled to 10°C, or lower, to prevent further reduction in pH. If the product is fermented in containers, no breakage of curd is necessary, and fermentation may take 16–18 hr. If sour cream is fermented in a tank, stirring, pumping, and packing cause a slight deterioration of its structure, or thinning (1). To minimize deterioration, ice water or coolant may be introduced to the jacket of the vat or processor. The agitator should only be rotated several turns, at intermittent intervals, throughout the time required to cool the product (3). Sour cream usually keeps for 3–4 weeks and the pH is generally 4.2–4.4.

After the curd is formed, at about pH 4.5, sour cream may be packaged cold or warm. If cooled in the vat, a TA of 0.70 or a pH of 4.5 must be attained prior to agitation. Stirred cold sour cream can be pumped from the vat through a grid insert in the pipeline into the hopper of a mechanical filler. Alternatively, the warm sour cream can be pumped through an in-line grid or an in-line homogenizing valve assembly at 170 to 690 kPa (25–100 psi) into a packaging machine hopper. This assembly is located in the sanitary pipeline at the discharge side of a variable-capacity sanitary positive pump. The warm or cold sour cream must be packaged immediately in 114–228 g consumer containers or 2.5–4.5 kg polyethylene-lined tins (40). The containers should be cooled overnight to 3–5°C and held for 24–48 hours before distribution to observe for bloats (gas production) or other rejects.

Table 6 Troubleshooting Guide for Sour Cream Production

Observation	Explanation	Solution
Curdy appearance (nonhomogeneous)	Agitation or movement of weak coagulum. Incomplete mixing of culture inoculum. Incomplete hydration of dry ingredients.	Avoid agitation until coagulation is complete. Completely mix culture into mix base. Allow complete hydration of dry ingredients.
Gassy appearance (small bubbles or pockets)	Contamination with CO_2-producing microorganisms may also be associated with off-flavors.	Avoid contamination by using appropriate cleaning, sanitation, and inoculation procedures.
Dull appearance (lacking gloss)	Excessive use of stabilizer/emulsifier.	Reduce stabilizer/emulsifier to less than 0.4%.
Grainy texture (mealy mouthfeel)	Incompletely dissolved dry ingredients within the product base.	Incorporate NFDM, stabilizers and/or emulsifiers completely into mix prior to pasteurization and homogenization.
Lacks uniformity (nonhomogeneous)	Careless incorporation of ingredients and/or inadequate homogenization.	Blend dry ingredients completely prior to pasteurization and homogenization.
Too firm body	Overstabilized product base.	Reduce stabilizer/emulsifier to less than 0.4%. Modify processing techniques (homogenization and/or pasteurization).
Weak body (too thin)	Processing techniques not optimized. Lack of stabilizer/emulsifier.	Modify processing techniques (homogenization and/or pasteurization). Incorporate up to 0.35% stabilizer/emulsifier.

Source: Ref. 3.

Once containers are packed, they are transferred to a blast cooler until the temperature is lowered to about 5–7°C. The product can be held in a regular cooler until distribution. Boxes of packaged sour cream are palletized in a circulating air chillroom to maximize the cooling rate (40).

B. Troubleshooting

Even when strict controls have been enforced, sometimes product quality suffers. Table 6 outlines some of the defects that may be found in sour cream products, their probable cause, and possible ways to prevent or avoid these defects.

V. SUMMARY

Cultured dairy products, including yogurt and sour cream, continue to gain popularity in the United States as new products appeal to consumer tastes and consumers realize the potential health benefits. Manufacturers must maintain sanitary practices, formulate appropriately, and process consistently, to deliver what they promise: consistent, high-quality cultured dairy products to consumers.

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10
Fromage Frais

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I. INTRODUCTION

A. Cheese Classification

Hundreds of different cheeses are produced around the world. Officially, approximately 500 cheese types have been recognized (1). Several classification schemes have been developed to group these cheeses, and a good description of these classifications can be found in *Fundamentals of Cheese Science* (2). One system classifies cheeses based on hardness and spans the spectra from very hard, hard, semisoft, and soft. No category is developed for semisolid cheeses, and as such cheeses should not be described in this segment of the handbook, which covers semisolid dairy products. However, the author felt that several cheeses do belong in the semisolid dairy products group along with yogurt and sour cream. Thus, this chapter will describe one of these semisolid or "spoonable" cheeses: Fromage frais from France.

B. Characteristics of Fromage Frais

Fromage frais is a fresh acid-curd cheese that can be consumed immediately after production. Its name, fromage frais (translated: cheese fresh), clearly communicates to the consumer that it is a nonripened cheese, and freshness is a major attribute of this cheese. Fromage frais is very similar to the German cheese Quark, which has slightly higher solids content. In appearance it resembles yogurt, a resemblance that is amplified by the use of packaging similar to yogurt cups. The flavor is subdued, even bland, with a refreshing, mildly sour characteristic. Yet the product has a clear cultured flavor, which is not to be mistaken with acetaldehyde in yogurt. The mouthfeel should be smooth, rich, and homogeneous.

Compositions of fromage frais with different fat contents are outlined in Table 1. Declaration of fat content must be made on the label as percentage (g fat/100 g of product) (3). Some fromage frais is sold plain—no flavor/fruit is added. However, many versions exist with added fruit, herbs, flavors, and sweeteners. French legislation requires the cheese be made from pasteurized milk. Total solids content should be at least 15% when fat in dry matter is above 20% and at least 10% when fat in dry matter is below 20%. Permissible ingredients include sucrose, honey, natural flavors, fruit, pulp, juice, jam, fibers, and colorants. Nonnatural flavors to reinforce natural flavors may be added in low quantities. Sorbate or potassium sorbate are permitted in fruit mixtures as long as the quantity

Table 1 Examples of Composition (% w/w) of Fromage Frais with Different Fat Contents

Composition	Low fat	20% fat in dry matter	40% fat in dry matter
Total solids (%)	17	21	26
Fat (%)	.05	4.5	10.4
Protein (%)	13	13	12
Lactose (%)	3	3	3
Salt (%)	0.5–1	0.5–1	0.8–1

Source: Ref. 27.

remains below 2% (current dosage is generally less, 0.2%). Sanitary standards require below 10 coliforms per gram, below 1 fecal coliform per gram, below 10 *Staphylococcus aureus* per gram, and absence of *Salmonella* in 25 g.

C. Utilization

Fromage frais is frequently eaten as a dessert and may be served with fruit and sugar. The preflavored products are often sweetened (10–12%), packed in small cups (30 g/cup), and marketed to children. In addition, it can be served plain or with herbs, though the latter option is not currently used in France. Especially, the low-fat products provide an excellent source of proteins low in fat and calories.

Though fromage frais is traditionally a French cheese, it is now sold in many European countries (4). The sale of fromage frais is increasing in part due to the product attributes listed below. Its popularity with children is a reflection of the increased sale of flavored products, which generally are targeted toward children (Fig. 1)

Excellent nutritional quality—high protein, low fat (for low-fat varieties).
Mild taste well suited for children.
Higher viscosity than European yogurt and thus very convenient for young children learning to eat with a spoon.
Excellent safety record: the heat treatment, continuous post-fermentation processing procedures, and low pH minimize the potential for post-pasteurization contamination.
Low production costs due to highly automated processing.
High yield because several methods exist that lower protein content of the whey.

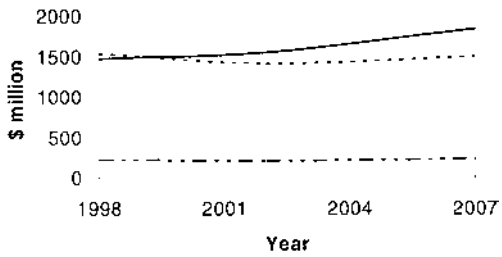

Figure 1 Retail value 1998–2007 (actual and predicted) in US$ for fromage frais and Quark sold in western Europe. Top (solid line) = "fruit flavored"; middle (dotted line) = "plain"; bottom (mixed line) = "savory". (Courtesy of Katya Fay, Euromonitor International 2002.)

Ease of product diversification: different fat contents, flavors, sweeteners, and fruit can be incorporated into the same cheese base prior to packaging.

Ease of packaging: the product is packaged on equipment similar to yogurt fillers and can be packaged into a wide variety of cup shapes and sizes.

II. FERMENTATION

Mixed strains of mesophilic acid producers are utilized, principally *Lactococcus (Lc) lactis* ssp *lactic* and *Lc lactis* ssp *cremoris*. In addition, aroma producers such as *Lc lactis* subsp *lactis* biovar *diacetylactis* (*Cit*$^+$ *Lactococci*) and *Leuconostoc mesenteroides* ssp *cremoris* are included.

The acid producers are homofermentative and convert lactose into lactic acid. Lactic acid is the basis for the clean sour flavor of the product and is also responsible for coagulating the cheesemilk. The conversion of lactose into lactic acid has been extensively studied and is well defined (5,6) Lactose is transported into the bacterial cell by the phosphoenolpyruvate-phosphotransferase lactose transport system and by lactose permease. During the transport, galactose is phosphorylated and transformed into glyceraldehyde-3-phosphate through the D-tagatose-6-phosphate pathway and into glycolysis. The glucose moiety is phosphorylated and metabolized through the glycolytic (Embden-Meyerhof) pathway (6,7). The overall homofermentative reaction can be described in simple terms (8):

$$\text{Lactose} + 4\text{ADP} + 4\text{H}_3\text{PO}_4 \rightarrow 4\text{Lactic acid} + 4\text{ATP} + 3\text{H}_2\text{O}$$

Aroma producers do not follow the Embden-Meyerhof pathway because they lack aldolases. Instead they convert lactose into a variety of end products such as lactic acid, ethanol, acetic acid, and CO_2. More important is their ability to metabolize citrate (9,10). Milk contains approximately 1600 mg citrate per kilogram of milk. Citrate permease transports citrate into the bacterial cell. Citrate is converted to oxaloacetate, pyruvate, and eventually diacetyl (11), which is an important flavor compound in fromage frais.

Continued conversion into the flavorless compound acetoin is possible (12) and depends on residual citrate levels, pH, and enzyme activity, which is strain specific.

III. FORMATION OF ACID COAGULUM

The mesophilic fermentation slowly converts lactose to lactic acid, which leads to increased acidity of the cheesemilk. The acid development causes several physico-chemical changes in the milk (13), primarily based on the conversion of casein from micellar form to a casein matrix. It is generally recognized that colloidal calcium phosphate connect and bridge casein submicelles within the casein micelle. Most or all of the colloidal calcium phosphate is solubilized by the time that acidification is complete around pH 4.5 (14,15). This solubilization initially leads to dissociation of individual caseins from the casein micelles (maximum around pH 5.2–5.4) (16,17). However, as the acidification continues, the proportion of serum casein decreases and is practically absent at the isoelectric point. This reversal is thought to be due to the reduction of surface charge on the proteins as they approach their pI, coupled with the increased concentration of calcium ions, which neutralizes charged regions. In this environment, soluble casein reattach onto the surface of casein micelles.

Gelation is thought to progress through several stages, which have been classified as pregel, and stages 1–4 (18) and acidification and gel formation are considered concurrent processes. Increases in viscosity are observed starting around pH 5.2–5.3 as casein aggregates become evident (19). These initial aggregates consist of altered casein micelles, small dissociated submicelles, and micelles with reattached dissociated casein. As pH reduction continues, the aggregates touch and form a three-dimensional network (13). Hydrophobic-, hydrogen-, and electrostatic interactions are all involved in the structure of the final gel (20).

Rennet may be added once fermentation has commenced. Rennet cuts κ-casein and releases the hydrophilic glycomacro peptide, which leads to lower surface charges on the casein micelles. In addition, fewer caseins dissociate from the micelles, causing less changes in shape and size of the original micelles. These factors initiate casein aggregation at a higher pH, which provides additional time for a firmer network to form.

Obtaining the correct gel structure is essential for product characteristics such as mouthfeel, consistency, and water-binding capacity (21). For example, a slowly formed gel consists of a highly ordered casein network with high water-holding capacity, whereas a quickly formed gel (fast fermentation) results in a coarse network, with large serum pockets, which may lead to syneresis (22). Coarse casein network, accompanied by syneresis, has also been attributed to higher gelation temperature (23). In contrast, protein standardization leads to a stronger, firmer gel (17) with smaller serum pockets, which lower the tendency for syneresis (24,25). Severe heat treatment (above required pasteurization temperature) increases product viscosity because heat denatured whey proteins (α-lactalbumin and β-lactoglobulin) will associate with κ-casein on the micelle surfaces and self-aggregate as well, thereby becoming part of the protein network instead of being dispersed in the serum phase (26).

IV. PROCESSING

A typical process for fromage frais is outlined in Fig. 2 (27). This process is highly automated and is used in large commercial processes. Each processing step is described in further detail below. It should be kept in mind that many alternative and more traditional processes are utilized in smaller plants.

A. Centrifugation and Thermization

A thermization or heat shock treatment (65°C, 15 to 30 sec) generally follows the initial centrifugation that separates raw milk into skim milk and cream. This heat treatment is not a legal pasteurization but suffices to destroy most spoilage bacteria. In general, this heating increases process flexibility by allowing more time for protein standardization without deterioration of skim milk quality.

B. Protein Standardization

While cheeses traditionally require casein-to-fat standardization, fromage frais is prepared from skim milk and only protein standardization occurs. The reasons for standardizing protein content are threefold: (a) to ensure constant protein content throughout the year, (b) to increase cheesemaking capacity of the processing line, (c) to promote a firm coagulum (see Sec. III). The standardization can be done by adding protein or by concen-

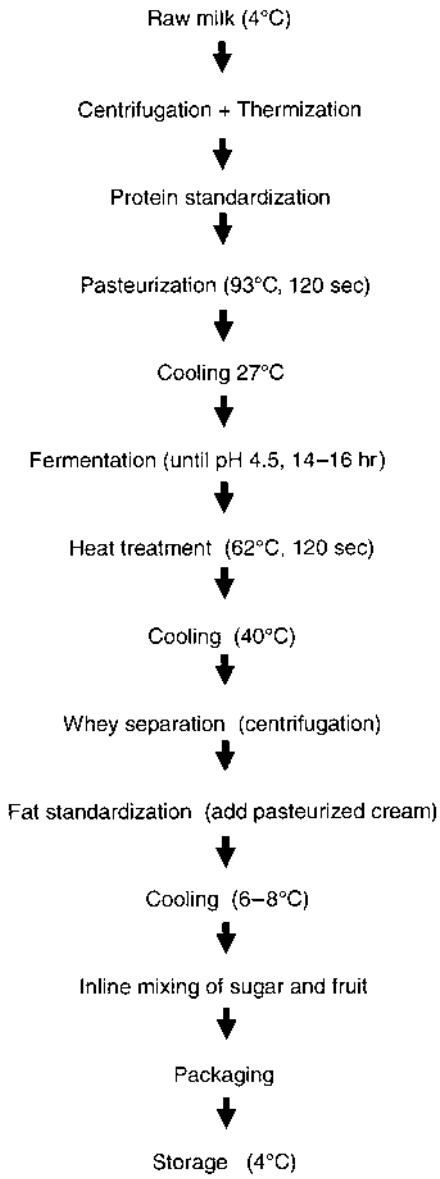

Figure 2 Processing of fromage frais using curd centrifuge. (From Ref. 27).

trating the skim milk. Typically, proteins are added as nonfat milk powder or milk protein concentrates. It is important that time is available for the added powders to become properly hydrated. Hydration rates are increased if proper mixing equipment such as a triblender is utilized. The subsequent pasteurization and cooling also promote hydration.

Concentration of skim milk can be achieved through membrane technology or by evaporation. The choice of method affects composition of the standardized skim milk. Ultrafiltration (UF) retentates contain fewer low-molecular-weight compounds such as minerals and lactose than evaporated concentrates because low-molecular-weight compounds are removed in the UF permeate. Compositional differences can have profound

influence on cheese characteristics and must be controlled by changing processing parameters such as rennet addition and fermentation rate and extent (28).

C. Pasteurization

It is important to pasteurize as quickly as possible to prevent growth of psychrotrophic spoilage bacteria. Pasteurization conditions are well above minimum requirements for destroying potential raw milk pathogens. The severe heat treatment creates a higher viscosity of the coagulum, in part because whey proteins are heat sensitive and denature at severe heat treatment. Denatured whey proteins are retained in the casein network, thus leading to increased cheese yield. Westfalia obtained a patent (29) in 1977 for the Thermo-Quark process, which is frequently used in fromage frais processing. The patent covers the process of pasteurizing cheesemilk to 90–95°C for 2–3 min followed by fermentation and then a second heat treatment of the coagulum at 60°C for 1–2 min. The advantage of this process is a decrease in protein content of the whey by up to 50%.

Following the holding tube, the milk is cooled to the appropriate fermentation temperature in the regeneration section of the pasteurizer. Typical fermentation temperatures are around 25–27°C.

D. Fermentation

The quantity of starter cultures added depends on the activity of the culture, the incubation temperature, time available for fermentation, and the sensory characteristics expected of the finished product. In general, 0.5–3% culture is added. Approximately 90 min after addition of starter culture, the milk/coagulum reaches a pH of 6.3 and rennet is added (.5–1 mL single strength/100 L cheesemilk). Fromage frais is a fresh acid-curd cheese and can be made without rennet. However, rennet firms the coagulum and subsequently minimizes the loss of shattered casein curd into the whey. If severe heat treatment has been utilized to denature whey proteins, it is beneficial to increase rennet quantity because κ-casein is protected by the denatured whey protein. However, compared to traditional rennet cheeses such as Cheddar, the rennet addition to fromage frais is low.

The fermentation takes 14–16 hours and is complete when pH reaches 4.5, whereupon the coagulum is well mixed to assure a smooth texture. Fermentation time above 18 hr indicates slow cultures and could pose a safety risk. In contrast, overly fast fermentation may contribute to syneresis as described above (Sec. III). Fermentation time is a function of starter activity as well as fermentation temperature.

E. Heat Treatment

The coagulum is heat treated at around 62°C for 120 sec to improve whey separation in the subsequent step. In addition, this heat treatment destroys some bacteria and enzymes, thus increasing shelf life of the final product. The disadvantage of this heat treatment is that lactic acid bacteria from the starter culture are destroyed as well. It is estimated that only 10% of lactics survive the treatment (27).

F. Separation of the Whey

Syneresis is required to increase solids content of the cheese. Following fermentation the coagulum physically retains moisture within serum pockets. To permit moisture release it

is necessary to break the aggregated protein strands around serum pockets. Breaking the strands releases moisture, and the broken strands will reaggregate into a more compact structure, thus encapsulating less moisture (13).

Traditionally, whey separation in cheesemaking is obtained by cutting the curd, cooking, and draining the whey. The automated and continuous whey separation step in fromage frais processing utilizes a centrifuge to mechanically remove whey (Fig. 3). The coagulum is cooled to around 40–44°C before entering the centrifuge. Within the centrifuge, curd is fed into the center of the bowl and through the distributor into the rising channels of the disc stack (27). The centrifuge separates the coagulum into curd (δ = 1.05) and whey (δ = 1.02). Curd centrifuges are specifically developed for viscous products and have capacities from 1000 L/hr to 10,000 L/hr. The centrifuge is cooled by circulating cold water through the hood. Solid content of the curd is determined by the feed rate as well as the nozzle discharge capacity. It is possible to install different diameter nozzles when changes in solids content are required. Solid-nonfat contents range from 13 to 24%, dependent on equipment and the cheese produced. Constant solid content is obtained by having a homogeneous coagulum composition and constant feed rate to the centrifuge. A well-installed centrifuge can keep variations in total solids within $+/-0.05\%$. Besides variations in composition, failing to optimize operation of the centrifuge can lead to defects such as sandy mouthfeel. The whey should be clear and free of curd particles. Whey can be tested by placing a sample in a lab centrifuge and centrifuging at 3500 rpm for 9 min (27). If suspended particles are present, it is necessary to optimize variables such as temperature, feed rate, pretreatment, and solid content of the coagulum. For nonfat coagulums, the whey constitutes the light phase, which moves toward the interior while the curd is pushed outward. Highfat coagulums require different centrifuges because in this case the light phase is the fat-containing curd and the heavier whey is pushed outward.

To optimize yields, an ultrafiltration system can be connected to the whey stream to collect whey proteins, which are then reintroduced into the curd (30). However, the Thermo-Quark/Thermo-soft method, which denatures whey proteins, is often considered a more efficient process for yield improvement. Another approach to increasing yield is to minimize nonprotein nitrogen by selecting a starter culture with low proteolytic activity.

Ultrafiltration can also be used for concentrating milk solids in place of the curd centrifuge. The UF system can be installed either before or after fermentation. In the former scenario, only retentate is fermented because the concentration step occurs before fermentation. Concentration of cheesemilk up to final total solid content is utilized in the production of other cheeses as well, such as feta, Camembert, and Brie. This process was developed by Maubois, Mocquot, and Vassal in 1969 (31). To obtain the correct calcium balance and ultimately a comparable coagulum, it is necessary to slightly preacidify the milk prior to ultrafiltration. Traditionally, whey contains high calcium content because solubilization of colloidal calcium phosphate occurring during fermentation shifts calcium from the casein fraction to the serum fraction. UF retentate retains colloidal calcium phosphate unless the milk is acidified prior to filtration. Furthermore, UF retentate contains all whey proteins, which lead to a weak coagulum unless the proteins are denatured by severe heat treatment (26).

As stated above, UF can also be performed after fermentation. In this case, the calcium balance will likely be correct because the fermentation has caused solubilization of colloidal calcium phosphate. When installed after fermentation, the UF system basically replaces the curd centrifuge as indicated in Fig. 4. It has been stated that this process gives a higher yield than the thermoquark method (32) and provides higher process flexibility because higher fat coagulums can be processed (33) (Fig. 5).

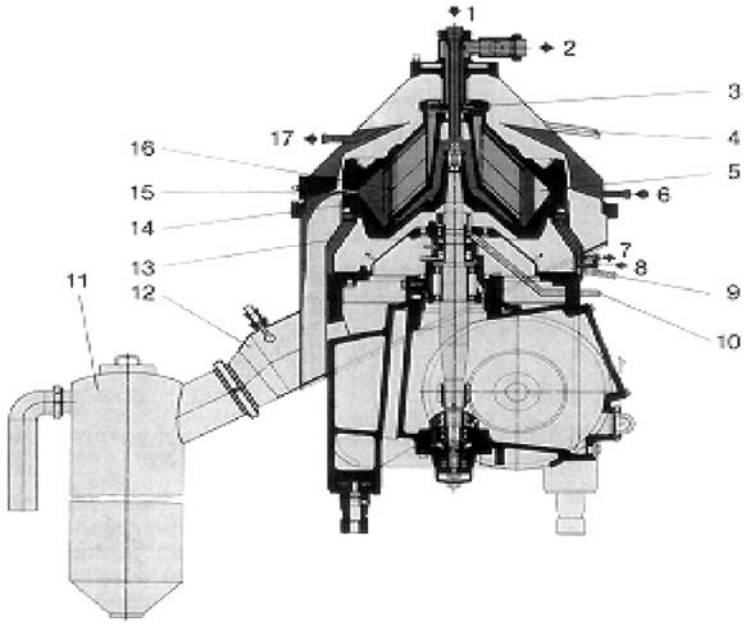

Figure 3 Curd separator type KDB 30-02-076. 1: Feed; 2: Discharge—whey; 3: Centripetal pump—whey; 4: Hood overflow; 5: Segmental insert; 6: feed—hood cooling; 7: Discharge, concentrate collector, and brake ring cooling; 8: Feed, concentrate collector, and brake ring cooling; 9: Frame drain; 10: Sterile air/CIP connection; 11: Cheese hopper; 12: Concentrate collector; 13: Brake ring, cooled; 14: Rising channels; 15: Nozzles; 16: Disc stack; 17: Discharge, hood cooling. (From Ref. 27.)

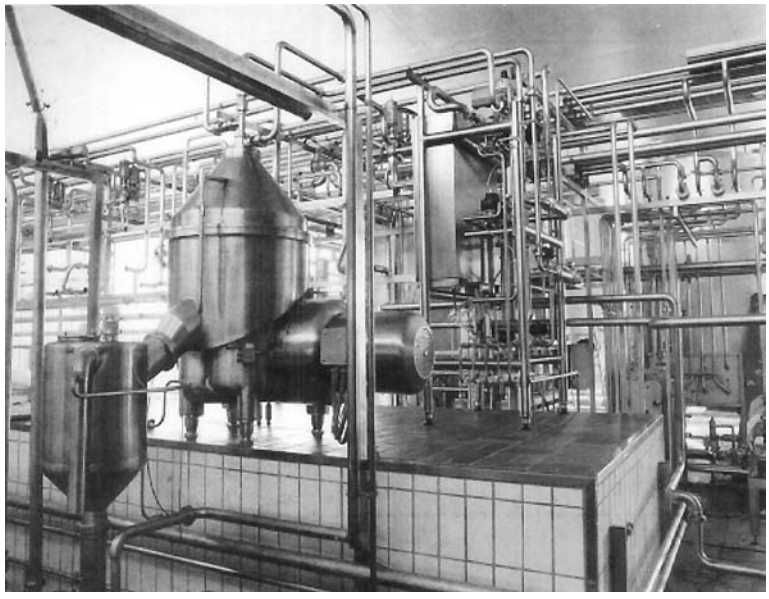

Figure 4 Thermo-Quark cheese line with KDB 30 separator. (From Ref. 27.)

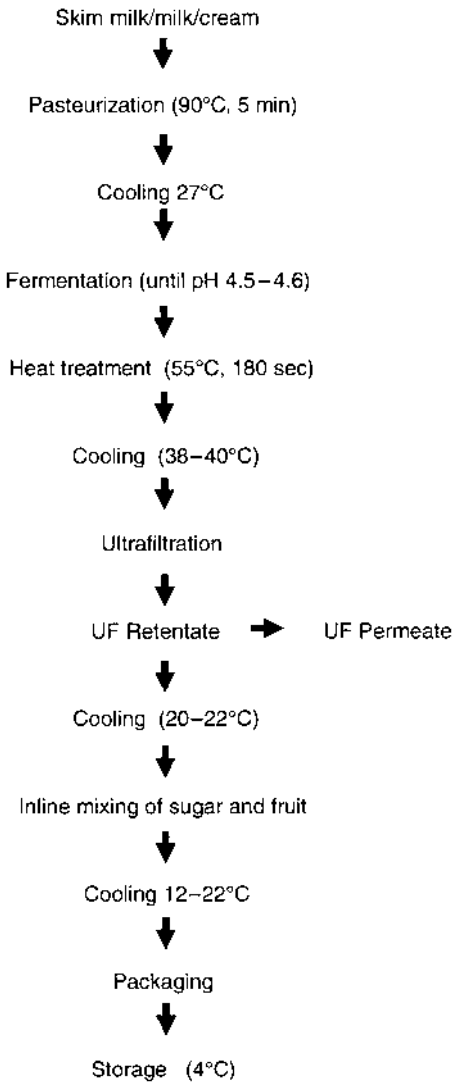

Figure 5 Processing of fromage frais using ultrafiltration system. (From Ref. 33.)

G. Cooling

Incorporation of refrigerated cream has a cooling effect; however, additional cooling is required. Tubular heat exchangers are frequently utilized for cooling because of the gentle effect on product viscosity. Likewise, positive-displacement pumps must be utilized at all times following curd separation to assure minimal product abuse.

H. Fruit Ingredients

The low pH of fromage frais makes it sensitive to yeast and mold growth (34). Traditionally, these contaminants were often introduced with the fruit. Dairy processors re-

sponded to this challenge by demanding high microbiological standards from fruit suppliers. The risk can be controlled through addition of sorbate (permitted by European legislation), which is effective against a wide range of yeast flora (35). However, processing plants that practice high levels of sanitation, process control, ultraclean packaging rooms, filtered laminar air flow, and so forth can produce fromage frais without using sorbate or any other preservative.

I. Packaging

Fromage frais is packaged in cups ranging in size from 0.03 kg to 1 kg. The fillers are similar to yogurt fillers and work at high capacity. Packaging materials are often polystyrene or polypropylene and the cups are either preformed by injection molding or thermoformed prior to filling.

Table 2 Product Defects and Potential Remedies

Defect	Cause	Remedial Action
Unclean flavors	High bacterial content of raw milk	Improve quality of raw milk, decrease raw milk storage temperature and storage time.
	Postpasteurization contamination	Improve cleaning and sanitation.
	Excessive levels of potassium sorbate	Decrease product storage and distribution temperatures
		Decrease concentration of potassium sorbate
Yeast and mold contamination	Addition of contaminated fruit products	Improve quality of fruit products added
		Add sorbate
Bitter off flavors	Proteolysis	Improve quality of raw milk to lower concentration of bacterial heat stable proteases
		Lower concentration of rennet or change to different rennet product
Grainy texture	Product pH too close to isoelectric point	Lower pH of product
Syneresis	Incorrect gel formation	Decrease rate of fermentation, increase protein content, increase pasteurization temperature
	Product abuse during storage and distribution	Increase cold storage time before distribution. Decrease storage and distribution temperature. Decrease physical movement (shaking, bumping) of products during transportation

V. SKIM MILK REQUIREMENT

Lehmann, Dolle, and Bucker (27) provide a formula for calculating the skim milk requirement for production of 1 kg of low-fat fromage frais.

$$S_m = \frac{DM_Q - DM_w}{DM_{SM} - DM_w}$$

where

S_m = kg skim milk per kg low-fat fromage frais
DM_Q = % dry matter in the fromage frais
DM_w = % dry matter in the whey
DM_{SM} = % dry matter in the skim milk

VI. SHELF LIFE

Typical shelf life is around 24 days (maximum 30 days to retain the label "frais" [fresh]). Limiting factors are microbiological deterioration (yeast, mold, spoilage bacteria), development of bitter off-flavors, whey separation, and grains. Defects and potential remedies are summarized in Table 2.

VII. SUMMARY

Fromage frais is a popular cheese because of its nutritional qualities, multiple diversification opportunities, and excellent safety record. Its make-procedure lends itself to automated, high-yield processes. These advantages translate into increased market shares on the European market. It is certainly possible that fromage frais eventually will become a standard item in the U.S. portfolio of dairy products.

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11

Semisolid Cultured Dairy Products: Packaging, Quality Assurance, and Sanitation

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I. PACKAGING OF SEMISOLID FERMENTED DAIRY PRODUCTS

A. Introduction

Packages play an important role in quality conservation of semisolid fermented dairy products. Packaging is very important and demanding for perishable cheese products such as fresh (cottage) cheeses. Well-designed packages help to extend shelf life, improve quality, and ensure safety of products. These benefits can be used to reach a higher level of flexibility in the distribution chain and to gain a better position in the market competition. As for any food product, the most important roles of a package include:

Protection of product from environment factors, including dirt or other foreign materials, microorganisms, gases (O_2), and light; by preventing contamination, packaging serves a public health functions as well as reduces losses due to spoilage.
Reduction of moisture loss from the surface, thus increasing economic return.
Prevention of physical deformation.
Provision of relevant information to consumers—e.g., name and origin of the product, ingredients, nutritional information, instruction for use, and expiration date.

To meet these important roles for a package of semisolid fermented dairy products, including yogurt, sour cream, and fresh cheeses, the following aspects should be considered (1,2):

1. The packaging must successfully contain the product at low cost.
2. For most of these products, easy access with spoon or knife is essential. This indicates the use of wide-mouth containers for this product category.
3. A good advertising billboard must be provided by the package, which is one reason why the plastic construction are almost always pigmented.
4. Packaging materials must be highly resistant to attacks by fats and oils, proved a good barrier to moisture loss, and free of migration of plasticizers.
5. In most cases, a barrier to UV light is required to avoid oxidative rancidity development in these high-fat-content materials.

6. It is particularly important that the packaging materials do not contribute undesirable flavor or odor chemicals that could be easily detected or cause harm, because many of these products have delicate flavors.
7. Packaging has to provide convenience to consumers (e.g., single serve vs. institution bulk).

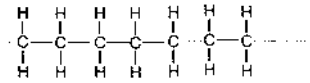
Design of packaging system to meet the above requirements involves several aspects, including selection of correct packaging material, package geometry and construction, and packaging equipment. This chapter emphasizes only packaging materials and package geometry and construction—not the equipment aspect.

B. Packaging Materials, Geometry and Construction

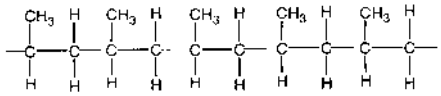
Semisolid fermented dairy products, including yogurt (drinkable yogurts are excluded in this chapter), sour creams, and fresh cheese, are always sold refrigerated and have a relatively short packaging shelf life of about 30 to 45 days under refrigeration. Products in this group are soft and thus require semirigid or rigid packaging. Containers are the most popular package type for these products. The container must be acid-resistant, prevent the loss of flavor volatiles, and be impermeable to oxygen, because the presence of oxygen can encourage the growth of contaminating yeasts and molds. Materials usually used for the manufacture of the containers include HDPE (high density polyethylene), PP (polypropylene), PS (polystyrene), PVC (polyvinyl chloride), and PVDC (polyvinylidene chloride). Chemical structures of these polymers are illustrated in Fig. 1, and their permeability to water and gas are listed in Table 1. These materials meet essential strength and low-cost requirements and are also inert, in the sense that they contribute no off-odors or off-flavors to the products. Except for the occasional use of clear PET (polyethylene terephthalate), containers are almost always pigmented, with the purposes of protecting the product from visible light, concealing the uneven clarity of these plastics, making the print more attractive, and adding additional market features by using color.

The finished containers can be cartons, tubs, or cups and can be manufactured in any shape or design to meet product requirements and attract consumer attention. Two different techniques are usually used to manufacture the plastics cups: injection-molding or thermoforming. The injection-molding process involves melting the plastic in an extruder and using the extruder screw to inject the plastic into a mold, where it is cooled. After the cup is formed, it is ejected from the mold. This type of container has relatively thick walls and is characterized as a rigid cup. In the thermoforming process, the polymer is delivered to the dairy plant in the form of a continuous roll, one end of which is fed into the first section of the product filling/packaging machine. The polymer sheet is heat-softened and formed into or around a mold, so that the unit container is formed immediately prior to filling with the product. This packaging system is referred to as a form/fill/seal operation and is used mostly for yogurt packages. In the thermoforming process, the yogurt cups have a relatively lower wall thickness than those produced by the injection-molding system and are classified as semirigid cups (2,4).

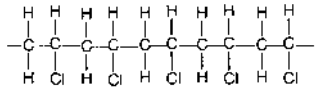
The lids of the containers are also usually injection-molded or thermoformed using materials such as HIPS (high impact polystyrene) or LLDPE (linear low density polyethylene) sheetstock and are snapped on, rather than sealed, to provide easy opening/closing. The tamper-evidence rings that surround the lip of the top are usually made from a 50–75 μm PVC shrink film. The film is heat-shrunk in place after the package is filled and lidded (5). Paper-based lids have also been used lately for dairy applications such as yogurt cups



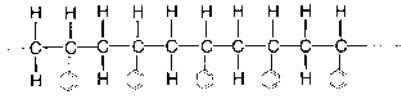
Low Density Polyethylene (LDPE)



Polypropylene (PP)



Polyvinyl Chloride (PVC)



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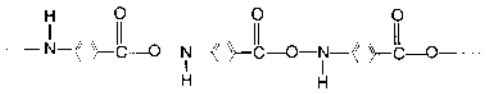
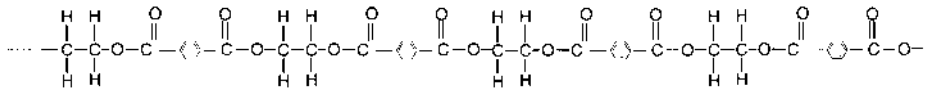
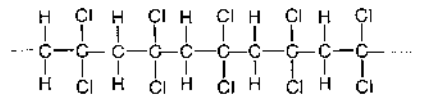


Nylon 6



Polyethylene Terephthalate (PET)



Polyvinylidene Chloride (PVDC)

Figure 1 Chemical structure of the polymers commonly used for manufacture of containers used for semisolid fermented dairy products.

Table 1 Permeability Data for Polymers Used Most Commonly in Fermented Dairy Products

	P ($(cm^3 \cdot mm)/(s \cdot cm^2 \cdot cm \cdot Hg)$)						
	N_2	O_2	CO_2	H_2O, 90% RH	P_{O2}	P_{CO2}	
Polymer	30°C	30°C	30°C	30°C	P_{N2}	P_{N2}	Nature of polymer
LDPE	19	55	352	800	—	—	Some crystallinity
HDPE	2.7	10.6	35	130	3.9	13	Crystalline
PP	—	23	92	680	—	—	Crystalline
Unplasticized PVC	0.4	1.2	10	1560	3.0	25	Slightly crystalline
PS	2.9	11	88	12000	3.8	30	Glassy
Nylon 6	0.1	0.38	1.6	7000	3.8	16	Crystalline
PET	0.05	0.22	1.53	1300	4.4	31	Crystalline
PVDC	0.009	0.053	0.29	14	5.6	31	Crystalline

LDPE: Low-density polyethylene; HDPE: High-density polyethylene; PP: Polypropylene; PS: Polystyrene; PVC: Polyvinyl chloride; PET: Polyethylene terephthalate; PVDC: Polyvinylidene chloride.
Source: Ref. 3.

and soft cheese. Paper-based, aluminum-free peelable lid material is prepared with a barrier coating that permits easy peeling without tearing. The lid can provide a barrier against oxygen, water vapors, and odors while offering excellent printing capabilities (6). Another lid technology is a patented polyethylene recloseable tamper-evidence cap for yogurt and cultured dairy products introduced by Portola Packaging, Inc. (San Jose, CA). Using Autoprod (Autoprod Packaging Equipment, Clearwater, FL) heat-form closing technology traditionally used for milk cartons, the cap uses a mechanical external ring with a pull tab for 8-oz dairy containers. To open, a consumer grasps the pull tab and tears away the locking ring.

In the following sections, specific packages for yogurt, cottage cheese, and sour cream, with highlights on the new technologies, will be briefly discussed.

1. Yogurt

Robinson and Tamime (7) and Tamime and Robinson (4) describe the types of packaging materials and constructions for yogurt in great detail. Basically, three types of packaging containers are used for fluid-type and semisolid yogurt: rigid, semirigid, and flexible units. The rigid units include recyclable glass bottles, and metal cans or aluminum foil laminated pouches. Semirigid units are normally manufactured from different plastics and made as tubs. This type of package is the most popularly used today. The flexible units are either in the form of plastic sachets or paper cartons, and used mostly for fluid-type yogurt.

In the United States, yogurt was packed in wax-or polymer-coated paperboard containers with recloseable covers and thermoformed containers. Now, most products are automatically filled in PS tubs and covered with aluminum foil and a polymer cap. Recently, a couple of new packaging designs specially targeted for portability have helped boost yogurt sales. Yoplait Go-Gurt is packaged in a three-sided tube-shape pouch; no spoon is required to eat the yogurt. The pouch is made of Polyester/LDPE/LDPE-based sealant layer and produced on a form/fill/seal machine. The tubes are packaged in solid bleached-sulfate cartons in multiples of eight (Fig. 2). A spoonable yogurt in a cup sporting the Danimals brand was introduced in 1994 and then relaunched in January 1999 (Fig. 2). Part of the relaunch involved new package graphics. The small white high-density PE bottles are extrusion-blown in custom molds. Lidding is aluminum foil. The operation forms the roll-fed foil into a cap complete with pull tab. It heat-seals the foil to the bottle

Figure 2 Typical containers for the packaging of yogurt.

finish and crimps the foil around. Shelf life of the refrigerated product is about the same as any other refrigerated yogurt—30 days.

Efforts to reduce packaging waste and provide environment-friendly operation have been emphasized lately. Glass was rejected by the yogurt industry because the costs of transporting the heavy material outweigh the benefits, although glass is widely recycled and made from recycled material. The energy (fossil fuels) used for manufacture and transport of glass containers exceeds the energy used for plastic containers. Paper yogurt containers are welcomed by consumers, but packaging materials of this type are coated with a thin layer of plastic, making them a combination container and not recyclable. FDA prohibits the use of recycled material in contact with food. Polypropylene containers have become the best option in yogurt package to assure product quality, with the least amount of material, fossil fuel, and environmental impact. One of the most beneficial characteristics of polypropylene is that it can be made with thinner walls while maintaining the same structural integrity, thus significantly less plastic than HDPE can be used. The quart containers are over 30% lighter today than they were 10 years ago. By using this material instead of HDPE, one yogurt manufacturer in 1998 alone prevented the disposal of over 85 tons of plastic. Added environmental protection was achieved through decreased air emissions and resource depletion associated with the manufacture and distribution of the packaging. In addition, polypropylene is manufactured without the use of chlorine, thus eliminating the hazards of deadly dioxin releases during manufacture and incineration, which occurs with certain other plastics.

2. Fresh and Cream Cheeses

Protection against light, oxygen, and loss of moisture is the basic requirement for packages of fresh and cream cheeses. The packaging must provide protection against light transmission. The oxygen in fresh cheeses may be present in the cheese as a result of the processing techniques used (e.g., centrifugation), occupy the headspace inside the package, or permeate the package over time. Because of the high water content of fresh cheeses, they are very sensitive to dehydration. Therefore, they need to be protected from moisture loss by barrier packages (8).

Genuine vegetable parchment or greaseproof paper was frequently used in the past to package fresh cheese. Paper coated with paraffin or PVC/PVDC (polyvinylidene chloride) copolymer is still sometimes used in the form of a banderole (e.g., for packaging an unripened cheese intended for consumption within a short time) (9). Today, fresh cheeses, such as cottage cheese, are often packaged in rigid packages because of their soft and semiliquid texture and ready loss of moisture. Vacuum-formed polystyrene (PS) tubs are now used almost exclusively for consumer packaging of cottage cheese. The PS tubs offer superior

protection from fat absorption and breakage. Their mechanical and chemical properties have been significantly improved by copolymerization with, for example, butadiene. By co-extruding or extrusion coating with PVC or PV/PVDC copolymer, PS barrier properties have also been improved. In addition, the PS tubs can be pigmented with TiO_2 to provide a better barrier to light. The use of PVC is favored due to its inertness and its impermeability to water and gas, as well as its extraordinary resistance to fats (9).

As it is for other tubbed dairy products, tamper evidence is an important package requirement for cottage cheese. PP shrink film that surrounds and totally encloses the tubbed product is suggested for this purpose. This approach also provides additional moisture barrier function and significantly increases the shelf life of the product. However, this approach has not been widely adopted because it is more costly, new investment in shrink packaging equipment is required, and the truncated cone geometry of the tub is a difficult shape to shrink-wrap without leaving unsightly excess film on the package—the so-called dog-ear problem. Vinyl shrink bands have become a popular method for meeting this requirement for cottage cheese.

In addition to plastic tubs, aluminum foil with a thickness of 7–20 μm has been used for packaging fresh cheeses: the thicker foils (15 to 20 μm) are formed into containers, either rectangular in shape with straight walls, or cylindrical in section with corrugated or pleated sides. The corrosion of the aluminum must be prevented, which is usually done by applying a suitable enamel or by laminating with LDPE or PP. Otherwise, aluminum lactate will form due to the lactic acid from the whey coming into contact with the aluminum wall and attack the walls of the container, sometimes perforating them (9). For the manufacture of rectangular containers, combinations of aluminum foil with plastic and paper can be formed from a reel of materials, filled with (generally pasteurized) fresh cheese and heat sealed. Other combinations of paper, wax, and aluminum are also used (7).

Another new packaging technology for fresh cheeses is the application of modified atmosphere packaging (MAP) and processing to improve microbiological safety and to extend shelf life (10–17). Modified atmosphere packaging is a technology of flushing food packaging with a premixed gas mixture—usually elevated CO_2 and reduced O_2, balanced with N_2 (to reach total 100% gases in the headspace)—before sealing. Carbon dioxide, a known antimicrobial agent, has been shown to inhibit the growth of some psychrotropic organisms that contribute to the deterioration of refrigerated dairy products. Significant amounts of CO_2 occur naturally in milk. Unfortunately, it quickly dissipates during modern processing. A process for directly adding CO_2 into the cream dressing before mixing it with the cheese curd was developed. With moderate barrier packaging, shelf life might be 60 days or more (18–20); i.e., another 2–3 weeks of shelf life can be added to cottage cheese by packaging it in a carbon dioxide–rich atmosphere. The cottage cheese suffers no deleterious effects when the correct gas mixture is used. The CO_2 concentration must be limited to 40% (with nitrogen as the balance) to avoid undesirable flavors and collapse of the package during storage. Table 2 provides recommended gas mixture for different types of cheeses using MAP technology. In addition, the CO_2 must be present throughout the depth of the cheese: it is not sufficient to simply flush the headspace. This is achieved most simply by bubbling the gas through the creaming mix. The correct temperature control at 5°C is crucial to obtain the full extra 2–3 weeks of shelf life (22).

3. Sour Cream

Similar to the packages used for yogurt and cottage cheese, plastic tubs made of PS or PP are the most popular packages for sour cream. With the rising popularity of meals featuring sour cream as toppings as well as sour cream–based dips, a few changes have come along.

Table 2 Recommended Gas Mixture for Different Types of Cheeses Using Modified Atmosphere Packaging (MAP) Technology

Product	Hard cheeses	Hard cheeses (sliced)	Fresh/soft cheeses
MAP Gas mixture	80–100% CO_2 + 0–20% N_2	80–90% CO_2 + 10–20% N_2	20–40% CO_2 + 60–80% N_2
Gas volume/ Product weight	50–100 ml 100 g cheese	50–100 ml 100 g cheese	50–100 ml 100 g cheese
Typical shelf-life			
Air	2–3 weeks	2–3 weeks	4–14 days
MAP	4–10 weeks	4–10 weeks	1–3 weeks
Typical material[a]	OPA/PE–PA/ PE PA/PE	OPA/PE–PA/ PE PA/PE	OPA/PE–PA/PE
Typical machine	Deep-draw machine Horizontal flow-pack	Deep-draw machine Horizontal flow-pack	Deep-draw machine Horizontal flow-pack
Storage temp.	4–6°C	4–6°C	4–6°C

[a] PVC is being replaced by APET or PS/EVOH, and PVDC is being replaced by EVOH or OPA.
Source: Ref. 21. Table used with permission of Linde AG, Germany. MAPAX is a registered trademark of AGA AB, member of the Linde Group.

For example, squeezable bottles were developed specifically to meet consumer "topping" needs. Another example is in the 6.5-oz (184 g) deli line: the ordinary-looking old container has been replaced by a ramekin-style container inside a sleeve. Printed on the inside of the new sleeve are recipes, serving suggestions, coupons, and other cross-merchandising materials. In addition, modified atmosphere packaging technology has also been used to help preserve product freshness.

C. Future Trends in the Packaging of Semisolid Fermented Dairy Products

The trends that will influence the direction of packaging development for the semisolid fermented dairy products are similar to these affecting other food products discussed elsewhere. This will mainly include:

Consumer convenience: the squeezable bottle may be a harbinger of this trend.
Small packages fit the increasing trends toward single servings; yogurt has already gone a long way in this direction as it becomes the most popular snack item.
Environmental factors toward degradable packaging.
Profitable niche markets for differentiated products: packaging, particularly plastic packaging, is an important tool used by manufacturers to create and dominate such markets.

II. QUALITY ASSURANCE AND SANITATION IN MANUFACTURING SEMISOLID FERMENTED DAIRY PRODUCTS

A. Quality Control vs. Quality Assurance

Quality is commonly defined as "achieving agreed customer expectations or specifications." That is, the customers define the quality criteria needed in a product. To meet this

standard, the manufacturer puts in a quality control system to ensure that the product meets these criteria on a routine basis. Controlling quality of foods may be achieved by:

Inspection of raw materials to ensure that high-quality ingredients are used
Checking on the process to ensure that the weights of the ingredients and processing conditions, such as temperature and time are correct
Inspecting the final product to ensure that high-quality products are sent to the consumers

The quality-control approach is focused on the process, whereas the problems that customers may face can also occur elsewhere in the production and distribution chain. Quality assurance systems take a much wider view of what is involved in satisfying customers' needs, and focuses on the prevention of problems and not simply on their cure. Curing problems is expensive and quality can't be "inspected into" a product (23).

Quality assurance can be defined as a strategic management function that establishes goals and provides confidence that these measures are being effectively applied (24). Quality assurance is the set of systematic and preestablished actions necessary to provide adequate confidence that a product or a service meets the given quality requirement (25). A quality assurance approach includes the whole production and distribution system, from the suppliers of important raw materials, through the internal business management to the customer. Quality assurance systems should be documented in a simple way to show who has responsibility for doing what and when. The focus of quality assurance is prevention, and this should mean that action is taken to meet a specification and prevent failures from occurring a second time. This is done by planning, management action, and agreements with key suppliers and other people in the distribution chain (23).

Quality assurance can only be accomplished when staffs are well trained and motivated. Workers are normally well aware of the causes of most problems, and when quality assurance is used properly they can resolve most quality problems within their control. It is the responsibility of business owners to ensure that the quality assurance system, together with any necessary equipment and information, are available to the workers to allow them to exercise this control. A range of problem-solving techniques can be provided for process workers to use when trouble arises during production. These simple techniques are tried and tested. They involve problem identification, analysis of the cause, suggestions for solutions and implementation, and feedback methods. These techniques allow the operators more control over their work and allow problems to be prevented rather than solved. More advanced statistics-based methods can be used for sampling plans and process optimization, but these are beyond the scope of this book.

B. Food Safety Concerns in Fermented Dairy Products

Fermented dairy products were originally developed as a means of preserving raw milk in times of excess production and are generally considered to be relatively safe foods. However, several reported outbreaks of foodborne illness have been associated with these products. Organisms such as *Listeria monocytogenes*, *Salmonella*, *Brucella melitensis*, and *Escherichia coli* O157:H7 have all been involved in cheese-associated outbreaks, causing severe illness and some deaths. *Listeria monocytogenes* can cause meningitis and blood infections and abortion in pregnant women; some types of *Salmonella* can cause blood infections and may result in long-term illness such as reactive arthritis; *Brucella melitensis* causes undulant fever. The disease persists for months if left untreated but is seldom fatal in humans. Toxin-producing types of *Escherichia coli* (including *E. coli* O157:H7) may cause hemorrhagic

colitis, hemolytic uremic syndrome, and renal failure, which may result in death, particularly in young children. Outbreaks have occurred when milk pasteurization was not carried out properly and when cheese made from pasteurized milk was subsequently contaminated with pathogenic microorganisms. More recently, an outbreak of *Staphylococcus aureus* enterotoxin in pasteurized milk resulted in over 14,700 cases in Japan (26).

The chances of foodborne pathogens gaining access to dairy products can be reduced by properly controlled pasteurization of milk. The U.S. Public Health Service developed a model regulation known as the Standard Milk Ordinance in 1924 for the important public health considerations related to milk production. During the past decade, problems associated with the sanitary control of milk and milk products have become extremely complex because of new products, new processes, new chemicals, new materials and new marketing patterns, which must be evaluated in terms of their public health significance. In 1978, the United States Department of Health Public Health Service and Food and Drug Administration developed "The Grade A Pasteurized Milk Ordinance (PMO)" to translate this new knowledge and technology into effective and practicable public health practices. The PMO was last revised in 1999 (27) and has been used as a guide to allow for uniform regulation of the milk industry. PMO includes information on:

Permits
Labeling
Inspection of dairy farms and milk plants
Testing, bacteriological, drug and pesticide residues
Construction of facilities
Water supply
Toilet and hand-washing facilities
Personnel health and hygiene
Utensils and equipment
Pasteurization equipment and procedures
Cleaning and sanitization
Insect and rodent control
Standard for grade A milk and milk products

Other prevention and control measures include employing Good Manufacturing Practices (GMPs), Standard Sanitation Operating Procedures (SSOPs), and Hazard Analysis and Critical Control Points (HACCPs). It is well recognized that HACCP can be an effective management tool for reducing the risk of food safety hazards in the food supply. HACCP is a commonsense approach that is based on a preventive rather than a remedial approach to food safety. The FDA has mandated HACCP for high-risk food industries, such as seafood and fruit and vegetable juices, and the USDA requires processors of meat and poultry to develop and implement HACCP programs. Many in the food industry not currently under HACCP regulations realize that a HACCP program makes sense and have voluntarily implemented HACCP programs. Although there are no federal regulations requiring HACCP for manufacturing of dairy products, programs are being initiated in some states, and HACCP appears to be the direction for the future. The International Dairy Foods Association (IDFA) recently published a HACCP plant manual for dairy products (28). The manual provides technical recommendations for development of a comprehensive HACCP food safety system for the entire dairy industry, including specific prerequisite program, preliminary steps, assessment of hazards, hazard analysis guideline, seven HACCP principles, and model HACCP programs covering different dairy products.

C. Good Manufacturing Practices (GMPs)

Good Manufacturing Practices (GMPs) are required by law for all food manufacturing companies. Information on GMPs can be found in the Federal Code of Regulations (CFR) section 21, part 110. GMPs are the foundation and prerequisites for all food safety and food quality programs. GMPs ensure the production of safe and wholesome food of high quality. GMPs are prescribed for four main areas of food processing as outlined in Table 3. The specific operation procedures in dairy plant can be found in the International Dairy Food Association (IDFA) HACCP plant manual for dairy products (28).

D. Sanitation Standard Operating Procedures (SSOPs)

Sanitation Standard Operating Procedures, or SSOPs, are specific step-by-step procedures needed for processes related to sanitation. It is mandatory for all food processing plants as written in the Federal Code of Regulations (CFR) Title 21, part 120.6. SSOPs need to address eight sanitation conditions (29):

1. Safety of water that comes into contact with food or food-contact surface, or is used in the manufacture of ice
2. Condition and cleanliness of food contact surfaces including utensils, gloves, and outer garments
3. Prevention of cross-contamination from insanitary objects to food, food-packaging material, and other food contact surfaces, including utensils, gloves, and outer garments, and from raw product to cooked product
4. Maintenance of hand-washing and toilet facility
5. Protection of food, food-packaging material, and food-contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate, and other chemical, physical, and biological contaminants
6. Labeling, storage, and use of toxic compounds (cleaning solutions, pesticides, additives)

Table 3 Four Main Areas of GMPs in Food Processing

Areas	Description
1. Personnel hygiene	Knowing how and when to wash hands
	Understanding the importance of clean uniforms
	Proper use of hair and beard nets
	Policy on jewelry—minimal at best
	Policy on chewing tobacco, smoking, and eating
2. Building and facilities	Hand-washing stations
	Adequate lighting and ventilation
	Storage of ingredients (refrigerated and on pallets)
	Separation of raw ingredients from processed foods
	Pest management program
3. Equipment and utensils	Easily cleaned and sanitized
	Easily maintained
	Meet food grade standards
4. Production and process control	Time/temperature control charts
	Records on food ingredients
	Lot identification and coding
	Product weight controls

7. Control of employee health conditions that could result in the microbiological contamination of food, food-packaging materials, and food-contact surfaces
8. Exclusion of pests from the food plant

SSOPs will vary from facility to facility because each facility and process is designed differently. The step-by-step procedure of sanitation operations for dairy plants is described in great detail by Marriott (30) and in the IDFA's HACCP plant manual for dairy products (28). The following tasks were recommended by Marriott as components of a sanitation quality assurance system (30):

1. Clear delineation of objectives and policies
2. Establishment of sanitation requirements for processes and products
3. Implementation of an inspection system that includes procedures
4. Development of microbial, physical, and chemical product specifications
5. Establishment of procedures and requirements for microbial, physical, and chemical testing
6. Development of a personnel structure, including an organization chart for a quality assurance program
7. Development, presentation, and approval of a quality assurance budget for required expenditures
8. Development of a job description for all positions
9. Setup of an appropriate salary structure to attract and retain qualified quality assurance personnel
10. Constant supervision of the quality assurance program with written results in the form of periodic reports

1. Cleaning and Sanitation in Dairy Plants

Cleaning is the chemical or physical process of removal of visible soil or matter from a surface. Cleaning removes 90–99% of the hazards, but only sanitizing completes the job. Sanitizing is the process that results in reduction/destruction of microbes to a level that is of a safe and healthful nature. Cleaning and sanitizing are part of dairy plant sanitation programs that are included in Good Manufacturing Practices. Cleaned and sanitized equipment and building are essential to the production, processing, and distribution of wholesome dairy products. A good cleaning and sanitizing program can greatly improve the safety and quality of dairy products. Good sanitation programs must be established for:

All equipment used in production
Processing environment
Raw materials handling and storage
Processing hygiene and handling finished goods
Waste disposal
Employee hygiene and facilities
Finished product storage
Transportation

It is important to use appropriate cleaning compounds and equipment so that a sanitation program can be effectively administered in a shorter period of time and with less labor. Table 4 provides a guide to the most appropriate cleaning compound, cleaning procedure, and cleaning equipment for the dairy processing equipment. After cleaning, sanitizers should be applied to the cleaned surface to help destroy microorganisms. Of the many methods for sanitizing, those most frequently used in dairy plants are steams, hot water, and

Table 4 Optimal Cleaning Guide for Dairy Processing Equipment

Cleaning applications	Cleaning compound	Cleaning medium	Cleaning equipment
Plant floors	Most types of self-foaming, or foam boosters added to most moderate to heavy-duty cleaners	Foam (high-pressure, low-volume should be sued with heavy fat or protein deposits)	Portable or centralized foam cleaning equipment with foam guns for air injection into the cleaning solution
Plant walls and ceilings	Same as above	Foam	Same as above
Processing equipment and conveyors[a]	Moderate to heavy-duty alkalis that may be chlorinated or non alkaline	High-pressure, low-volume spray	Portable or centralized high-pressure, low-volume equipment; sprays should be rotary hydraulic
Closed equipment	Low-foam, moderate to heavy-duty chlorinated alkalis with periodic	Cleaning-in-place (CIP)	Pumps, fan or ball sprays, and CIP tanks

[a] Packaging equipment can be effectively cleaned with gel cleaning equipment.
Source: Ref. 31.

chemical sanitizers. Their applications in dairy processing plants are described in Table 5. In addition, the quality of water is critical because water is the major constituent of almost all cleaners. Because most plant water is not ideal, the cleaning compounds selected should be tailored to the water supply or should be treated to increase the effectiveness of the cleaning compound. It is especially important to reduce suspended matter in water to avoid deposits on clean equipment surfaces. Water hardness complicates the cleaning operation. Suspended matter and soluble manganese and iron can be removed only by treatment, whereas small amounts of water hardness can be counteracted by sequestering agents in the cleaning compounds used in the sanitation operation. If the water is hard or very hard, it is usually more economical to remove or minimize water hardness.

Table 5 Types of Sanitizing Agents and Their Applications in Dairy Plants

Sanitizer	Applications
Steam	Maintain steam in contact with product contact surface for 15 min when condensate leaving the assembled equipment is at 80°C.
	Used less in dairy plants because it is difficult to maintain at constant required temperature, energy costs are high, and steam application is more dangerous than other methods.
Hot water	Temperature of water has to be maintained at not less than 76.7°C and not more than 90.6°C for a time period of 30 sec.
	This technique is expensive because of required energy costs.
Chemicals	Chlorine, iodine-containing compounds, and quaternary ammonia are the three most commonly used chemical sanitizers.
	For large-volume, mechanized operations, sanitizer can be applied through sanitary pipelines by circulation or pumping sanitizing solution through the system.
	For small operations, equipment, utensils, and parts can be sanitized by submission in sanitizer solution.
	Closed containers (tanks or vats) can be effectively sanitized by fogging.

Source: Refs. 30 and 32.

2. Cleaning Equipment for Dairy Plants

The techniques for cleaning dairy plants vary depending on the plant size. The major portion of a large-volume plant is cleaned by the Cleaning-In-Place (CIP) system, which is the recognized standard for cleaning pipelines, milking machines, bulk storage tanks, and most equipment used throughout the processing operation. However, many small parts of equipment and utensils, as well as small containers, can be washed effectively in a recirculating-parts washer, called Cleaning Out of Place (COP) unit. The COP units contain a recirculating pump and distribution headers that agitate the cleaning solution. A COP unit can also serve as the recirculating unit for CIP operation.

The effectiveness of the CIP approach depends on the process variables, time, temperature, concentration, and force. Cleaning and sanitizing agents should be used exactly following the product directions. Rinse and washing time should be minimized to conserve water and cleaning compounds but should be long enough to remove soil and to clean effectively and efficiently. Temperature of cleaning solution should be as low as possible and still permit effective cleaning with minimal use of the cleaning compound. CIP operations in dairy plants are usually divided into two major categories: spray cleaning and line cleaning. The detailed description can be found in Marriott (30). A current trend has been toward modification of CIP system to permit final rinses to be utilized as makeup water for the cleaning solution of the following cleaning cycle and to segregate and recover initial product-water rinses to minimize waste discharges.

When COP equipment is used in dairy plants, the following steps are recommended (30):

1. A prerinse with tempered water at 37° to 38°C to remove gross oil
2. A wash phase through circulation of a chlorinated alkali cleaning solution for approximately 10 to 12 min at 30° to 65°C for loosening and eradicating soil not removed during the prerinse phase
3. A postrinse with water tempered to 37° to 38°C to remove any residual soil or cleaning compound

3. Cleaning Steps in Dairy Processing Plants

In "Item 9p—Milk Plant Cleanliness" of PMO, it is clearly stated that "All rooms in which milk and milk products are handled, processed or store, and/or in which containers, utensils or equipment are washed or stored, shall be kept clean, neat and free of evidence of insects and rodents". In Item 10p—Sanitary Piping, it states that "All sanitary piping, fittings and connections which are exposed to milk or milk products, or from which liquids may drip, drain or be drawn into milk or milk products, shall consist of smooth impervious, corrosion-resistant, nontoxic, easily cleanable material which is approved for food contact surfaces. All piping shall be in good repair. Pasteurized milk and milk products shall be conducted from one piece of equipment to another only through sanitary piping."

Eight cleaning steps in dairy operations are recommended. Their specific applications are described in Table 6.

E. Hazard Analysis and Critical Control Points (HACCPs)

One of the main building blocks used for developing a quality assurance system is the Hazard Analysis Critical Control Point (HACCP) system. HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical,

Table 6 Recommended Eight Cleaning Steps in Dairy Operations

Cleaning step	Application
Cover electrical equipment	Covering materials should be polyethylene or equivalent.
Remove large debris	This task should be accomplished during the production shift and/or prior to prerinsing.
Disassemble equipment as required	
Prerinse	Prerinsing can remove up to 90% of the soluble materials and also loosens tightly bound soils and facilitates penetration of the cleaning compound in the next cleaning step.
Apply cleaning compound	This step can be simplified through proper selection and use of processing and clean equipment, proper location of equipment, and reduction of soil accumulation. Further reduction of soil buildup is possible through use of the minimum required temperature for heating products a minimum amount of time; cooling product heating surfaces, when practical, before and after emptying of processing vats; and keeping soil films moist by immediately rinsing foam and other products with 40° to 45°C water and leaving it in the processing vats until cleaning.
Postrinse	This step solubilizes and carries away soil. Rinsing also removes residual soil and cleaning compounds and prevents redeposition of the soil on the cleaned surface.
Inspect	Inspection is essential to verify that area and equipment are clean and to correct any deficiencies
Sanitize	A sanitizer is added to destroy any residual microorganisms. By destruction of microorganisms, the area and equipment contribute to less contamination of the processed products.

Source: Ref. 30.

and physical hazards from raw material production, receiving and handling, to manufacturing, distribution, and consumption of the finished product. HACCP helps food processors compete more effectively in the world market, where HACCP is becoming the standard food safety assurance program. HACCP places responsibility for ensuring food safety appropriately on the food manufacturer or distributor. The features of HACCP include:

Focusing on identifying and preventing food safety hazards
Being proactive—preventive strategies are considered early in the process
Sound science basis
Forcing manufacturers to monitor their process continuously
Permitting more efficient and effective government oversight

Seven principles are included in developing a HACCP program. They are listed and briefly described in Table 7 (33).

GMPs and SSOPs are the prerequisite programs of HACCP and help to create an operating environment that allows the production of safe, wholesome food. All prerequisite programs should be documented and regularly audited along with the HACCP plan. In addition, product and process information, including product description, ingredient list, and process flow chart are the foundation for developing a HACCP plan. The IDFA's

Table 7 Seven HACCP Steps and Their Brief Description

7 steps of HACCP	Brief description
Step 1. Conduct a hazard analysis	Biological, chemical, and physical hazards are conditions that may pose an unacceptable health risk to the consumer. During the hazard analysis, all food safety hazards associated with each specific step of the manufacturing process are listed and those that are significant are identified.
Step 2. Identify critical control points	Critical Control Points (CCPs) are steps at which control can be applied so that a food safety hazard can be prevented, eliminated, or reduced to acceptable levels. Examples of controls include pasteurization, cooking, curing, acidification, or drying steps in a food process.
Step 3. Establish critical limits	All CCPs must have preventive measures that are measurable. Critical limits are the operational boundaries of the CCPs that control the food safety hazard(s). They might include pasteurization time/temperature limits, end cooking temperature, or product pH. Critical limits are set according to regulations in place (pasteurization of milk or cold holding temperatures at retail), experience with a product, research data, or in consultation with food safety authorities familiar with your product process. If critical limits are not met, the process is "out of control," and it is possible that food safety hazard(s) are not being prevented, eliminated, or reduced to acceptable levels.
Step 4. Monitor CCPs	Monitoring is a planned sequence of measurements or observations to ensure the product or process is in control (critical limits are being met). It allows processors to assess trends before a loss of control occurs. Adjustments can be made while continuing the process. The monitoring interval must be adequate to ensure reliable control of the process. Monitoring could include checking temperatures of a cooler twice per day, the use of a continuous temperature monitoring device on a pasteurizer, or a pH test.
Step 5. Establish corrective actions	HACCP is intended to prevent product or process deviations. However, should loss of control occur, there must be definite steps in place for handling the product in question and for correction of the process. Corrective action plans must be thought out and written as part of the HACCP plan, not determined at the time of the deviation. If, for instance, a cooking step must result in a product center temperature between 165°F and 175°F, and the temperature is 163°F, the corrective action could require a second pass through the cooking step with an increase in the temperature of the cooker.
Step 6. Record-keeping	The HACCP system requires the preparation and maintenance of a written HACCP plan together with other documentation. This must include all records generated during the monitoring of each CCP and notations of corrective actions taken. Usually, the simple record-keeping system possible to ensure effectiveness is the most desirable.
Step 7. Verification	Verification has several steps. The scientific or technical validity of the hazard analysis and the adequacy of the CCPs should be documented. Verification of the effectiveness of the HACCP plan is also necessary. The system should be subject to periodic revalidation using independent audits or other verification procedures.

Source: Ref. 33.

HACCP Plant Manual (28) describes specific guidelines for developing and implementing even steps of HACCP in dairy plants. Model HACCP programs for milk and milk products, frozen desserts including ice creams and yogurts, cheeses, and butter are provided.

Here are the practice guidelines for designing and implementing HACCP in dairy operations.

Set up a record-keeping system that is easy to use and easily accessed. Product records need to be reviewed before shipping.

Keep for your records minutes of discussions related to hazard evaluation, selection of critical control points, critical limits, potential monitoring activities, corrective actions, and so forth.

Begin the HACCP process early. Involve and use the experience and knowledge of people involved in each step, process or department.

Train employees so that they know what they are supposed to do and when they are supposed to do it.

Take a look at problems you have had in the past. Address those problems in your HACCP plan.

Activities covered by your SSOPs or GMPs probably do not need to be included in your HACCP plan.

Once you have developed your flow diagram on paper, go out into the plant and verify the flow diagram. Check product flow and employ related activities.

Once you have a HACCP plan draft, test it out in small segments.

Reassess your plan often in the beginning. Later you may need to reassess only 1–2 times per year.

Once your HACCP plan is in action and reassessment is made, document when and what changes are made to the plan. When changes are made, it is important that the plan and employee-related duties changes are made at the same time.

Establish a plantwide feedback system for continuous improvement.

Document in your plan where records are stored. Store HACCP records that need to be accessed separate from other company records.

Say WHAT will be done, HOW it will be done, and WHO will do it—then, DO WHAT YOU SAY.

F. Specific Prevention and Control Measures

In general, fermented dairy products are considered to have a low risk of causing food poisoning because they contain natural acids that prevent the growth of foodborne bacteria. Processing, such as fermentation (lowering pH) and heating (pasteurization), also reduces the risk of contaminating bacteria. However, the products can be infected with pathogens during processing or after. Processors should start with good-quality raw materials and have strict controls over hygiene and handling. The generalized process flow chart for fresh cheeses is shown in Figure 3 and a summary of the main quality assurance procedures for fresh cheese processing is shown in Table 8. The quality assurance procedures in the table are discussed in more detail in the following sections.

1. Raw Materials Handling, Storage, and Inspection

The basic ingredient of fermented dairy products is milk, hence ensuring high quality of incoming milk is essential to achieve high quality and safe products. Basic tests on the standard plate counts, coliforms, drug resdues, and phosphatase of milk are required for

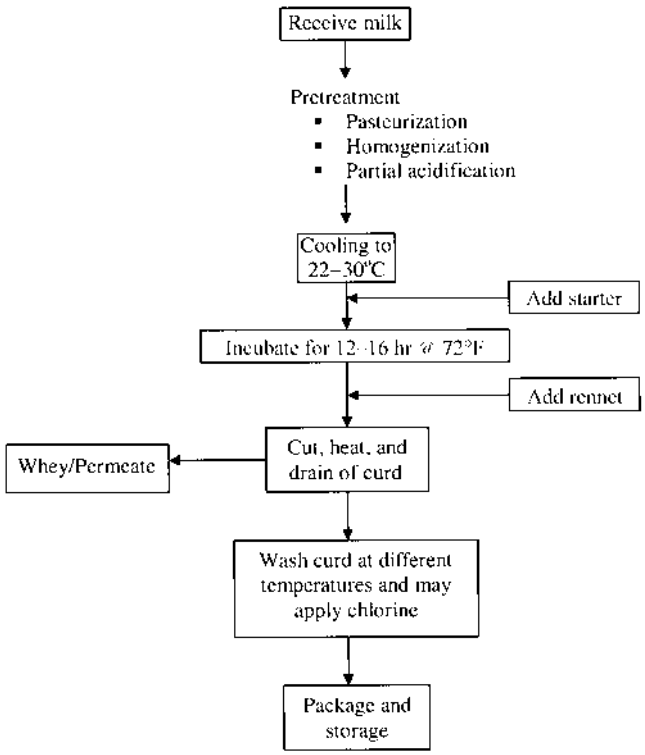


Figure 3 Generalized production flow chart of fresh cheese (cottage cheese).

determining milk quality. The PMO standards on grade A pasteurized milk are illustrated in Table 9. Other raw materials used in the production of fermented dairy products, including starter cultures, rennet, and packaging materials, have to be subject to examination, too. The standard methods for the examination of milk and milk products can be found in Marshall (34) and Fox et al. (35).

2. Milk Pasteurization

Milk pasteurization is a critical control point in a dairy processing HACCP program. An appropriate heating temperature and time is essential to destroy or prevent the growth of undesirabl microorganisms. Table 10 lists PMO recommended minimum pasteurization temperature and time combinations for milk and milk products. The basic pasteurization principle is that "every particle of milk or milk product be heated to at least a minimum temperature and held at the temperature for at least the specified time in properly designed and operated equipment." In addition to the basic operation procedures for high-temperature short-time (HTST) milk pasteurization suggested by PMO, other requirements are:

Calibrate thermometers in the pasteurizing unit to ensure the correct temperature readings.

Reject milk not heated to the required temperature for the required time, and redirect it through the pasteurizer again.

Check at startup that the flow diversion valve on continuous flow pasteurizing units are operating correctly.

Table 8 Quality Assurance Procedures for Fresh Cheese Processing

Stage	Process activity	Control point
Raw material handling and storage	Receive milk from farms, and purchase starter cultures and rennet supplies.	Arrival temperature of milk should be <10°C, and storage at <6°C. Starter cultures and rennet should be stored at cold and dry places.
Raw material inspection	Record amount and quality of milk and other raw materials.	Specification of milk quality based on the total colony counts, inhibiting substance, composition, etc., microbiological examination, activity tests, and absence of contamination on starter cultures.
Ingredient formulation/ batch preparation	Weigh and mix ingredients	Training in accurate weighing and keeping records of ingredients used.
Processing control	Pasteurize milk, adding start culture, rennet, curd setting; cut, drain, and wash curd to make required products.	Preparation of processing schedule and training of operators to ensure: control of temperature and time of heating correct amount of starter culture and rennet added at the correct time in the processing Establish standards for operator hygiene and schedule for cleaning of equipment and processing room
Packaging	Fill product into packages, seal and label. Pack into distribution boxes.	Establish specifications for package quality, labels, and fill weight. Implement inspection, check-weighing, and recording procedures.
Storage and distribution	Store finished product. Dispatch products in required amounts to retailers or customers.	Control store room temperature and implement cleaning schedule and stock rotation procedures. Ensure records are kept. Establish inspection and recording procedures to ensure that customers receive the correct product in the amount specified.

Regularly monitor the results of phosphatase testing of pasteurized milk to ensure that the heating plates of the HTST pasteurizer do not leak.

Maintain, correctly calibrate, and service equipment regularly.

3. Packaging, Storage, and Distribution

Packaging, storage, and distribution should be performed in such a way that product is protected against contamination through the following measures.

Packaging of milk and milk products shall be done at the place of pasteurization in a sanitary manner by approved mechanical equipment.

Cottage cheese, dry-curd cottage cheese, and reduced-fat or low-fat cottage cheese may be transported in sealed containers in a protected, sanitary manner from one plant to another for creaming and/or packaging.

Seals on lids should have close and continuous contact with the container rim. Plastic and glass seals should not be contaminated with product or else the seal will leak.

Table 9 Chemical, Bacteriological, and Temperature Standards for PMO Grade A Milk and Milk Products

Grade A raw milk and milk products for pasteurization, ultra-pasteurization or aseptic processing	Temperature	Cooled to 7°C (45°F) or less within 2 hours after milking—provided, that the blend temperature after the first and subsequent milking does not exceed 10°C (50°F).
	Bacterial limits	Individual producer milk not to exceed 100,000 per milliliter prior to commingling with other producer milk. Not to exceed 300,000 per milliliter as commingled milk prior to pasteurization.
	Drugs	No positive results on drug residue
	Somatic cell count[a]	Individual producer milk: Not to exceed 750,000 per milliliter
Grade A pasteurized milk and milk products and bulk shipped heat-treated milk products	Temperature	Cooled to 7°C (45°F) or less and maintained thereat.
	Bacterial limits[b]	20,000 per milliliter or gram[c]
	Coliform[d]	Not to exceed 10 per milliliter—provided, that in the case of bulk milk transport tank shipments, shall not exceed 100 per milliliter
	Phosphatase[d]	Less than 350 milliunits/L for fluid products and less than 500 for other milk products by the Fluorometer or Charm ALP or equivalent.
	Drugs[b]	No positive results on drug residue.
Grade A aseptically processed milk and milk products	Temperature	None.
	Bacterial limits	Refer to 21 CFR 113.3(e)(1)[e]
	Drugs[b]	No positive results on drug residue

[a] Goat milk, 1,000,000.
[b] Not applicable to cultured products.
[c] Results of the analysis of dairy products that are weighed in order to be analyzed will be reported in number per gram (see the current edition of the *Standard Methods for the Examination of Dairy Products*).
[d] Not applicable to bulk-shipped heat-treated milk products.
[e] 21 CFR 113.3(e)(1) contains the definition of "Commercial Sterility."
Source: Ref. 27.

Packaged products should be stored in cardboard (paperboard) boxes on pallets or shelves that keep the boxes off the floor of the storeroom. Boxes can be stacked on top of each other provided that the combined weight of the stack does not crush the bottom box. The storage room should be cool and dark, with good ventilation to maintain a flow of air.

Attention to the construction of a processing area and storage rooms should be paid carefully. The details of building sanitary design and layout should be applied equally to processing and storage rooms. In particular, windows should be screened against insects and the structure of the roof and walls should prevent rats and birds from gaining entry.

Table 10 PMO Recommended Minimum Pasteurization Temperatures and Times for Milk

Temperature	Time
63°C (145°F)[a]	30 min
72°C (161°F)[a]	15 sec
89°C (191°F)	1.0 sec
90°C (194°F)	0.5 sec
94°C (201°F)	0.1 sec
96°C (204°F)	0.05 sec
100°C (212°F)	0.01 sec
Provided, that eggnog shall be heated to at least the following temperature and time specifications:	
69°C (155°F)	30 min
80°C (175°F)	25 sec
83°C (180°F)	15 sec
Provided further, that nothing shall be construed as barring any other pasteurization process which has been recognized by the Food and Drug Administration to be equally efficient and which is approved by the regulatory agency.	

[a] If the fat content of the milk products is 10% or more, or if it contains added sweeteners, the specified temperature shall be increased by 3°C (5°F).
Source: Ref. 27.

A management system should be developed to monitor which products are in storage, to control stock rotation and record their destination for delivery. This is particularly important when a processor produces a range of products, as it is very easy for slower-selling products to be hidden at the back of a store and go beyond their "sell-by" date. Simple records should be kept by storekeepers to show which products and materials are transferred into and out of the storerooms.

G. Sampling Procedures and Methods

Sampling for quality evaluation is another important aspect in quality assurance system. The sampling procedures and methods must be defined at the time of samples, and be appropriate and statistically valid to ensure that the results are accurate and provide complete information. Sampling procedures must include identifying and collecting only representative samples, maintaining samples at correct temperature, protecting samples from contamination and damage, and keeping samples in their original container for lab analysis. Acording to PMO standards, milk and milk product samples shall be analyzed at an official or appropriate officially designated laboratory. All sampling procedures and required laboratory examinations shall be in substantial compliance with the most current edition of *Standard Methods for the Examination of Dairy Products of the American Public Health Association*. (34) and the most current edition of *Official Methods of Analysis of the Association of Official Analytical Chemists* (36). Such procedures, including the certification of sample collectors and examinations, shall be evaluated in accordance with the "Evaluation of Milk Laboratories, United States Public Health Service/Food and Drug Administration" (37).

For the group of semisolid fermented dairy products, the common lab analyses usually include:

Assessment of physical characteristics, such as consistency, texture (firmness), and viscosity
Chemical composition, including moisture, milk fat, and protein content
Microbial analysis in the final products; Table 9 provides the standards used in the regulations applied to milk and milk products
Sensory characteristics evaluated by sensory panel on color, flavor, taste, and texture evaluation

III. SUMMARY

Packaging is a very important element in the manufacture of semisolid fermented dairy products and significantly affects the shelf life, quality, and safety of the products. Critical aspects in the design of a packaging system include selection of appropriate materials that meet required barriers to moisture, gases, and light, and are environment-friendly; geometry and construction to protect product from environmental factors and physical deformation, and provide consumer convenience; and maintenance of low cost.

Quality assurance is a management system that controls each stage of food production from raw material to final product consumption for ensuring high quality and safety. GMPs, SSOPs, and HACCPs are the fundamental programs for a quality assurance system. The essential requirements for ensuring a high-quality fermented dairy product may be listed as:

Good quality of raw milk and ingredients
Correct processing conditions, including control of heating temperature and time for milk, inoculation time and temperature, pH, and water activity during processing
An active, well-balanced, and contaminant-free starter culture
Clean and well-sanitized plant and equipment
Well-trained employees
Correct packaging, handling, and storage of retail products at controlled temperature

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12
Principles of Cheese Production

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Cheese, which can be considered a nutritious and delicious concentrate of the valuable nutrients of milk in a stable form, has been produced since prehistoric times. The basic steps of modern cheese production are the same as the empirically based cheesemaking of thousands years ago. The present scientific knowledge on cheese production, however, has been developed mainly during the past 125 years.

I. BASIC PRINCIPLES OF CHEESEMAKING

A. Concentration

The first step in making cheese is coagulation of milk. Milk can be coagulated by the action of rennet enzyme or by acidification. The coagulum has a natural tendency to syneresis—contraction and expelling of whey (i.e., water and water-soluble compounds of the milk (Fig. 1). Larger particles such as fat globules and bacteria will be retained in the shrinking coagulum, the cheese curd. The syneresis can be accelerated by cutting up the gel and by heating. The resulting firm curd can be pressed and formed into cheeses of various shapes.

B. Preservation

The fresh curd contains valuable nutrients and can therefore quickly be spoiled by bacteria, yeasts, and molds if not preserved. The main factors contributing to preservation are low moisture content (concentration), acidification by adventitious or added lactic acid bacteria, and salting. Spoilage of the cheese by molds on the surface can be prevented by drying and cleaning the surface or by exclusion of oxygen by immersion in brine or by coating the cheese with wax or synthetic film material.

C. Ripening

Cheese may be consumed as fresh curd or it may undergo ripening by means of enzymes from the milk, from the rennet, and from microorganisms in the cheese or on the surface. During ripening, which may last for months, the organic solids of the cheese will gradually be hydrolysed and metabolized during which have the characteristic flavor, consistency, and texture of the cheese is being developed.

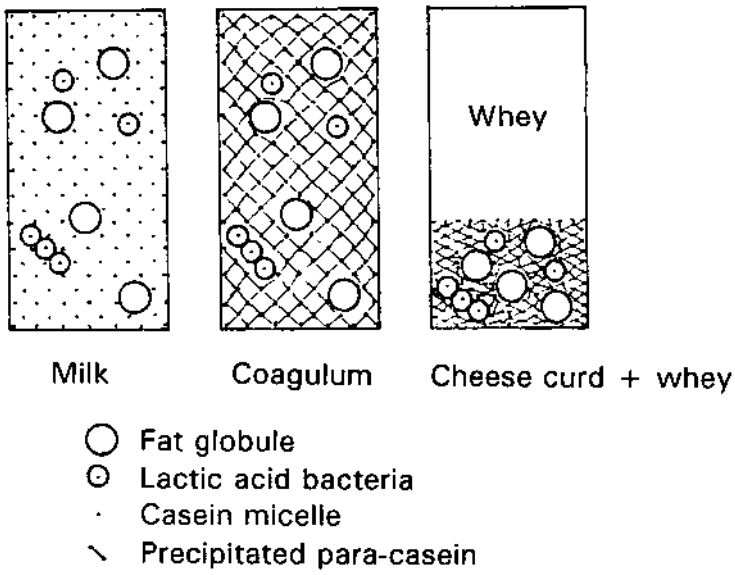

Figure 1 Outline of the structure of milk, rennet coagulum, and cheese curd. (From Ref. 1.)

In Table 1, some of the important types of cheeses are listed and grouped according to method for coagulation of the milk, their firmness, and the degree of acidification.

II. YIELD OF CHEESE

The majority of cheeses produced in the world are made from cow's milk. Substantial amounts of cheese, however, are also produced from milk of sheep, goat, and buffalo. Many of the milk components vary quantitatively, both between and within species. Estimated mean component figures are shown in Table 2.

A. Recovery of Milk Solids and the Yield of Cheese

When milk is coagulated, either by rennet or by acid, the caseins form a three-dimensional network enclosing all the other milk constituents. When the coagulum contracts, water and water-soluble constituents will be squeezed out, whereas the fat globules and bacteria will be retained in the casein network as outlined in Fig. 1.

1. Retention of Protein

The caseins, constituting about 75–80% of the milk proteins, are the basic structure-forming elements of coagulated milk and cheese curd. Casein is defined as those proteins of milk that become nonsoluble when milk is acidified to pH 4.6 (i.e., α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein, and γ-casein; see Table 3). The caseins in milk are aggregated in micelles 0.01–0.3 μm in diameter; and of the milk salts, much of the calcium, phosphate, magnesium, and citrate are associated with these micelles. When cheese is made by means of rennet enzymes,

Table 1 Cheeses Grouped According to Method of Coagulation, Firmness and pH Minimum

Coagulation of milk by	Firmness (moisture content on fat-free basis, MFFB)	Acidification, pH of young cheese	Examples of cheese types
Rennet	Extra hard (<51% MFFB)	5.10	Grana
	Hard	5.30	Emmentaler
	(49–56% MFFB)	5.10	Cheddar
	Semihard	5.20	Gouda, Danbo, Edam, Jarlsberg, Herrgård, Fontina, Svecia, Havarti, Tilsiter
	(54–63% MFFB)		Heated after acidification: Mozzarella, Kashkaval, Provolone
	Semisoft	6.10	Heated in whey: Halloumi
	(61–69% MFFB)	5.20	Heated after acidification: Soft Mozzarella
		5.15	Esrom, Limburger, St. Paulin
		4.70	Blue-veined mold cheeses: Roquefort, Gorgonzola, Stilton, Danablu
	Soft	4.60	White mold cheeses: Camembert, Brie
	(>67% MFFB)		Ripened in brine: Feta
			Milk salted: Domiati
Acid	Semisoft	4.50	Matured sour milk cheese: Gamal ost, Pult ost, Harzer Käse, Olomoucke tvaruzky
	(61–69% MFFB)		
	Soft	4.50	Fresh unmatured curd: Homogeneous curd: Tvorog, Quarg, Skyr Curd in grains: Cottage cheese
	(>67% MFFB)		
Made from cheese whey:			
Proteins of whey are precipitated by heating			Ricotta, Anari, whey protein cheese
Solids of whey concentrated by evaporation			Myse ost, whey cheese

Table 2 Estimated Mean Figures for Gross Composition of Main Solids of Milk (%w/w)

Species	Fat	Total protein	Lactose	Citrate	Ash
Cow	3.8	3.3	4.7	0.2	0.7
Buffalo	7.4	3.8	4.8	N.D.	0.8
Goat	5.6	3.8	4.8	N.D.	0.7
Sheep	7.4	5.5	4.8	N.D.	1.0

N.D., not determined
Source: Ref. 2.

Table 3 Typical Values for Distribution of Nitrogen Compounds (Protein Nitrogen and Nonprotein Nitrogen) of Whole Milk in the Nonsoluble versus Soluble (Whey) Components After Precipitation of Casein by Acid at pH 4.6 or by Rennet at pH 6.6

Percent of total nitrogen				
After precipitation at pH 4.6			After precipitation by rennet at pH 6.6	
	Nonsoluble		*Nonsoluble*	
Fat globule membrane protein (FGMP)[a]	1		1	Fat globule membrane protein (FGMP)
α_{s1}- and α_{s2}-casein	37		37	α_{s1}- and α_{s2}-casein
κ-casein	10	→ para-κ-casein 6.5 GMP 3.5	→ 6.5	Para-κ -casein
γ-casein[b]	3		3	γ-casein
β-casein[b]	27		27	β-casein
			1.5	Nonsoluble proteose-peptone
Total acid casein + FGMP	78		76	Total rennet casein + FGMP
	Soluble		*Soluble*	
			3.5	Glyco-macro-peptide (GMP)
Proteose-peptone (pp)[b]	3	→ Non-soluble 1.5[c] Soluble 1.5[c] →	1.5	Soluble proteose-peptone
Whey proteins	13.5		13.5	Whey proteins
NPN (nonprotein-N)	5.5		5.5	NPN (nonprotein-N)
Total acid-whey-N	22		24	Total rennet-whey-N

[a] When cheese is made from whole or part skimmed milk, the proteins of the fat globule membrane will be incorporated in the network of the precipitated caseins.
[b] In milk, a part of β-casein may be hydrolyzed by the action of the milk enzyme plasmin, resulting in γ-caseins (the more hydrophobic parts of β-casein) and proteose-peptones (the more hydrophilic parts).
[c] A part of the proteose-peptones, which all are soluble at pH 4.6, is precipitated with the caseins at pH 6.6. The partition of proteose-peptones on the soluble and the nonsoluble parts, after precipitation by rennet at pH 6.6, which in the table is set to 1:1, may be somewhat lower or higher (3,4).

coagulation takes place at about pH 6.6. The coagulation is brought about by enzyme-catalyzed hydrolysis of a single peptide bond of κ-casein:

$$\kappa - \text{casein} \rightarrow \text{para} - \kappa - \text{casein} + \text{glyco} - \text{macro} - \text{peptide (GMP)}.$$

The glyco-macro-peptide (GMP) constitutes about one-third of the mass of the κ-casein. The para-κ-casein component of the κ-casein remains as a part of the para-casein in the curd. The GMP is soluble and separates with the other soluble compounds (whey proteins, lactose, water-soluble vitamins, and the soluble salts) into the whey. Typical figures for the amount of the various groups of milk proteins as percent of total nitrogen, and their distribution on the nonsoluble part (in the cheese curd) and the soluble part (in the whey) after precipitation of casein with acid at pH 4.6 or with rennet at pH 6.6, are listed in Table 3; 76% is given as a typical value for retention of protein for rennet cheese. Variations in the relative amounts of the various protein fractions can cause actual protein retention values to vary from about 75 to 78% of total protein (N × 6.38).

Heat treatment of the milk can cause denaturation af the whey proteins. Heat-denatured whey proteins precipitate together with the caseins giving higher protein retention. *Higher* retention of protein can also be achieved by concentrating the protein by ultrafiltration. *Lower* retention of protein may be caused by inflammation of the udder or by growth of psychotropic, proteolytic bacteria during cold storage of cheese milk.

2. Retention of Fat and Bacteria

Fat globules with diameters of 0.5 to 10 μm will be incorporated into the casein network. Consequently, the retention of fat is high (85–95%).

For bacteria, the retention is of the same magnitude as for the fat globules.

3. Retention of Lactose

Lactose is dissolved in the water phase of milk and follows the whey out of the curd; hence, retention of lactose in curd is low, usually in the range of 3 to 5%, depending on the final moisture content of the cheese and on amount of water added.

4. Retention of Ash Compounds

Some of the minerals (K^+, Na^+, Cl^-) in milk are dissolved in the aqueous phase and these minerals separate with the whey. Others are present as colloidal particles integrated in the casein micelles (Ca^{2+}, Mg^{2+}, PO_4^{3-}). At the pH of fresh milk, these salts remain in the curd, but as the pH decreases during the syneresis these salts will gradually become dissolved.

Some of the phosphate is covalently bound in the casein molecules as serin-phosphate. This phosphate, which accounts for about 2 ½% of the mass of casein, will be included in the ash in analysis of both milk and cheese, a fact that should be taken into consideration in calculations on cheese yield. The figures found for retention of ash typically are about 35–40% for hard and semihard rennet cheese. For Cheddar cheese, with a higher degree of acidification during syneresis, retention of ash is lower—30–35%. For cheeses with acid precipitation of the casein at pH around 4.6, the retention of ash is low—10–15% of total ash.

5. Retention of Citrate

Most of the citrate in milk is in aqueous solution with 10% linked to the caseins together with calcium in the same way as with inorganic phophate. The retention of citrate will, therefore, depend partly on the whey content of the cheese and partly on the acidification during syneresis. For semihard cheeses, the retention of citrate is typically around 10%.

B. Calculation of Composition and Yield of Cheese

Table 4 shows an example of the calculation of yield of cheese and of composition of cheese. The example is based on typical figures for production of a semihard cheese (e.g., Danbo or Gouda).

III. MILK FOR CHEESE

For production of cheese, the milk has to be of good quality.

Table 4 Example of Calculation of Milk Solids in Cheese Curd, Yield of Cheese and Composition of Cheese, Based on Typical Figures for Composition of Cheese Milk and for Retention of Milk Solids in Cheese Curd

			Milk solids in cheese curd	
	In 1 kg milk	Retention in cheese curd	Gross calculated for cheese curd from 1 kg milk	Net calculated for cheese curd from 1 kg milk, with correction the part of the phosphate in the ash originating from serine-PO_4
Fat (standardized to give minimum 45% fat in dry matter of cheese)	30.0 g	0.92	27.60 g	27.60 g
Protein	34.0 g	0.76	25.84 g	25.84 g
Lactose (anhydride)	46.0 g	0.035	1.61 g	1.61 g
Ash	7.2 g	0.35	2.52 g	1.87 g
Citrate	1.8 g	0.10	0.18 g	0.18 g
Total solids in fresh cheese curd from 1 kg milk				57.10 g

Yield of cheese:

In addition to the milk solids, the cheese after salting includes extraneous NaCl. If the cheese after salting contains 43% moisture and 57% dry matter, of which 2% is NaCl and 55% is milk solids, the yield of cheese per kg milk will be:

$$57.1 \times 100/55 = 103.8 \text{ g}.$$

Then the composition of the salted cheese can be calculated:

Composition:		%
Fat:	$27.60 \times 100/103.8 =$	26.59
Protein:	$25.84 \times 100/103.8 =$	24.89
Lactose: (converted to lactic acid in cheese)	$1.61 \times 100/103.8 =$	1.55
Ash:	$2.52 \times 100/103.8 =$	2.43
Less the casein-PO_4 included in ash:		−0.62
Citrate[a]:	$0.18 \times 100/103.8 =$	0.17
Total milk solids in cheese		55.0
Plus extraneous NaCl		2.0
Total dry matter in cheese		57.0
Moisture in cheese[b]		43.0

[a] In cheeses like Danbo with citrate-fermenting starter bacteria, the citrate is converted within 1 to 2 weeks to volatile compounds such as carbon dioxide, diacetyl, and acetic acid.

[b] Under conditions of storage and ripening, the moisture content of the cheese (and the weight of the cheese) will decrease because of evaporation, unless the cheese is packed in synthetic film or covered with cheese wax.

A. Storage of Milk for Cheese

Unless the cheese is made shortly after milking, the milk should be kept cool till start of cheese production in order to prevent excessive growth of bacteria. During storage at low temperatures, the rennetability may be slightly reduced.

B. Pasteurization

Cheese is often made of raw milk, and it is a common assumption that cheese made of raw milk of good quality can be more rich in flavor. Some pathogenic bacteria, however, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, can survive in cheese for months, and in many countries pasteurization of milk for cheese is mandatory. Pasteurization at 72°C for 15 sec typically kills about 99% of the bacteria of the raw milk. Bacterial spores, however, will not be killed. About 95% of milk lipase will be inactivated at 72°C for 15 sec, a fact that may help to explain a part of the assumed richer flavor of raw milk cheese. Most of the whey proteins remain unchanged by pasteurization at 72°C for 15 sec, but at slightly more intensive heat treatment, the whey proteins begin to denature. Denatured whey proteins precipitate together with the caseins by the action of rennet, or by acid at pH 4.6, yielding a higher total retention of the proteins. For most cheeses, however, a heating greater than 72°C for 15 sec is normally not applied, because more intensive treatment implies certain drawbacks: slower renneting of the milk and a weaker coagulum; inactivation of the enzyme xanthine oxidase, which catalyzes a reduction of added nitrate to nitrite; and slower development of cheese flavor and texture during ripening.

C. Homogenization

For some types of cheese (e.g., Danablu, and Feta produced by ultrafiltration), homogenization may be applied in order to whiten the cream and to increase the water-binding capacity of the cheese curd. Homogenization also increases the lipolysis of the fat, provided active lipase is present.

D. Bactofugation and Microfiltration

These processes are widely used in the production of cheese types liable to damaging fermentation by the spore-forming bacteria *Clostridium tyrobutyricum* (e.g., Emmental and Gouda/Danbo cheeses). With *bactofugation* a large part of the bacteria, and more than 90% of the spores, can be removed from the milk. With *microfiltration* more than 99.9% of all bacteria and spores can be removed from the skim milk part of the milk. The cream of the milk then has to be heat-treated separately because the fat globules otherwise would block up the filter membranes.

In both processes, some of the bacterial spores follow the cream, adhering to fat globules. In order to produce cheese with a low number of *Clostridium tyrobutyricum* spores, the cream can be pasteurized at about 120°C for a few seconds, prior to mixing cream with skim milk from bactofugation or with permeate from microfiltration. Both the retentate from microfiltration and the bactofugate from bactofugation contain most of the spores from the milk and also a part of the casein, and for utilization of this casein, the retentate/bactofugate can be mixed with the cream before the heat treatment.

E. Ultrafiltration

By ultrafiltration, milk can be separated into a *retentate* in which fat, bacteria, casein micelles, and whey proteins are concentrated and a *permeate* containing water and lactose. Salts linked to the caseins in the micelles (most of the calcium and phosphate and a part of the citrate) will be concentrated in the retentate, whereas other salts will follow the water into the permeate. Ultrafiltration thus resembles the natural filtration process in the curd grains in traditional cheesemaking, in which the casein network acts as a filter—with the exception that the ultrafiltration membranes also retain the whey proteins. Concentrating milk for cheese by ultrafiltration is utilized in large scale of production of UF-Feta cheese, where the retentate, with about 40% total solids after addition of starter culture and rennet, can be *cast* directly into tins. Because the whey proteins are retained in the retentate, the yield of cheese by ultrafiltration is higher than in conventional cheesemaking. By ultrafiltrating of a part of the milk, the protein content of the cheese milk can be increased and standardized for conventional cheese production.

F. Standardization of Fat Content

Cheeses are usually classified by percent fat in dry matter (FDM) according to legal standards. The fat content of the cheese milk has to be adjusted according to the percent FDM desired and according to the protein content of the milk.

IV. ADDITIVES TO CHEESEMILK

A. Coloring Agents

The color of cheese is determined by the carotene color of the milkfat, which may vary with the seasons and with type of milking animals. Jersey milkfat, for example, is usually high in carotene whereas sheep milkfat is low. Where coloring is permitted, the color of cheese may be adjusted by means of annatto extract or carotene dye.

B. Salt

All cheeses are salted, either by addition of salt to milled curd after acidification and before molding, *or* by absorption of salt by diffusion after the cheese has been formed. A special case is Domiati cheese, where 8–15% salt is added to the *milk*, yielding coagulum and resulting cheese that is very soft.

C. Spore-Inhibiting Additives

For cheese of the Gouda/Danbo type, there is a risk of *Clostridium tyrobutyricum* spore growth during ripening, resulting in excessive gas production and the development of an unpleasant smell. The spores can be inhibited by means of nitrate added to the milk (0.1–0.2 g KNO_3/L); the nitrate is reduced to nitrite (NO_2^-), which inhibits the spores. This reduction is catalyzed by the milk enzyme xanthin oxidase, which is active in milk heated to about 72°C for 15 sec but inactive at a slightly higher heat treatment. Other possible additives for inhibition of spores are the enzyme lysozyme (from hen's egg white) and nisin (bacteriocin). The use of spore-inhibiting additives is legally restricted in cheese-producing countries.

D. Starter Cultures and Acidification

For most cheese varieties, starter cultures of lactic acid bacteria are added to the cheese milk. Where the curd is either not heated or heated to about 40°C, starters with mesophilic *Lactococcus* sp. and *Leuconostoc* sp. are used. If formation of carbon dioxide is undesirable (e.g., for Cheddar cheese, and for Feta cheese destined to be put in tins), mesophilic starters without citrate-fermenting *Leuconostoc* sp. and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (= *Lactococcus diacetylactis*) are utilized. In cheeses scalded at higher temperatures (e.g., Grana, Emmental), lactic acid fermentation is performed by thermophilic cultures of *Streptococcus thermophilus* and *Lactobacillus helveticus*.

Starter cultures can be local natural starters, or laboratory cultures from culture manufacturers either for propagation in the dairy or, as frozen or lyophilized starter concentrates, for direct inoculation in the cheese milk. The amount of starter culture added to the milk is typically about 1% for starter propagated in milk and about 0.01–0.05% for starter concentrates.

Slow acidification due to antibiotics in the milk or the development of bacteriophages can cause serious problems. Bacteriophages will not be destroyed by heat treatment of the milk at about 72°C for 15 sec, so there will always exist a risk of development of bacteriophages, along with the starter bacteria, in a cheese factory. Counteractive measures include careful cleaning and disinfection with chlorine or disinfectants with peracetic acid (hydrogen peroxide has no effect on bacteriophages), preventing any contamination of milk and curd with whey, propagation of starter culture in highly heated milk in a separate room, the use of starter cultures with good bacteriophage resistance, and if necessary, change of starter culture. Fast development of lactic acid bacteria is of utmost importance for cheese quality. For each type of cheese there is an optimal range for minimum pH; Table 5 gives some examples.

The effects of lactic acid bacteria are:

1. *Coagulation and syneresis*. The lactic acid promotes the action of rennet enzymes and the acidification of the curd increases syneresis. In the production of sour milk cheese (quarg, etc.) the milk is coagulated only by the acid produced by the starter bacteria.
2. *Inhibition of detrimental bacteria*. Lactic acid bacterial growth inhibits harmful bacteria by fermentation of all the lactose and by the formation of lactic acid. Some of the lactic acid bacteria also produce bacteriocins that may inhibit other bacteria.

Table 5 Optimal pH Minima for Various Cheeses

Cheese	pH minimum
Emmental	5.25
Gouda/Danbo group	5.20–5.25
Tilsiter/Havarti	5.15–5.20
Mozzarella	5.15–5.25
Cheddar	4.95–5.10
Feta, Danablu	4.65–4.7
Camembert	4.60–4.70

3. *Control of consistency*. The acidification controls the consistency and texture of the cheese. If the pH becomes too low, much of the calcium is dissolved from the casein network and the cheese will be brittle, less coherent. If the acidification is weak, little calcium is dissolved and the cheese mass will be rubbery. For semihard, sliceable cheese, the best consistency is obtained with a fresh cheese pH at about pH 5.2.
4. *Control of enzyme activity*. pH determines the activity of the various enzymes of the cheese during ripening.
5. *Taste*. Lactic acid is important for the fresh acid taste of young cheese.
6. *Low redox potential*. The lactic acid bacteria lower the redox potential to about −150 mV, yielding cheese that can be kept for a long time without the development of off-flavors due to oxidation of milkfat.
7. *Formation of proteolytic enzymes*. The lactic acid bacteria have proteolytic enzymes important for the breakdown of proteins during ripening. Intracellular peptidases, releaed after autolysis of the cells, are responsible for the formation of free amino acids; starter bacteria may also contribute to conversion of amino acids into various flavor compounds.
8. *Formation of CO_2 and diacetyl*: If the starter contains citric acid–fermenting bacteria such as *Leuconostoc* sp. and *Lactococcus diacetylactis*, the citric acid will be converted to acetic acid, diacetyl/acetoin, and CO_2.

E. The Course of the Acidification of Cheese

As described above, acidification is of fundamental importance for the quality of cheese. The velocity of the acidification is also important. A too-rapid drop in pH may cause the cheese to become too sour and too hard (increased syneresis); on the other hand, slow acidification gives the opposite effects and there will be a risk of growth of detrimental bacteria. Fig. 2, left, illustratesthe rate of acid development during the first 24 hours for a typical semihard cheese such as Gouda/Danbo, for a blue-veined cheese (Danablu), and for milk, all starting with inoculation with 1% mesophilic starter culture at 30°C. Fig. 2, right, shows the rise in pH for both types of cheese during ripening due to release of ammonia by the breakdown of proteins and by degradation of lactic acid. The faster increase in pH during ripening for Danablu, compared to Gouda/Danbo, is a result of very active proteolytic enzymes from the mold and aerobic degradation of lactic acid. In milk, acidification stops at about pH 4.4 because the bacteria are completely inhibited by lactic acid at low pH althoug there will still be surplus of nonfermented lactose. In cheese, the inverse situation prevails: the growth of the bacteria and the decrease in pH ends when all the lactose is used up, not because pH had become too low.

For the Gouda/Danbo cheese, the pH levels off at about pH 5.2; for the blue-veined Danablu cheese, pH continues to decrease until a minimum pH at 4.7. This difference is due to a difference in the lactose/buffering capacity ratio. The amount of lactose determines the amount of lactic acid formed, and the amount of buffers (i.e., protein and inorganic phosphate) determines the change in pH for a given amount of acid. In the young blue-veined cheese, compared to the Gouda/Danbo cheese, there is a higher moisture content, hence a higher content of lactose. At the same time, the blue-veined cheese has a lower content of buffers (protein and inorganic phosphate) than the Gouda/Danbo cheese.

A further important factor that contributes to higher pH minimum in Gouda/Danbo cheese compared to the blue-veined cheese is that for Gouda/Danbo, water is added to the

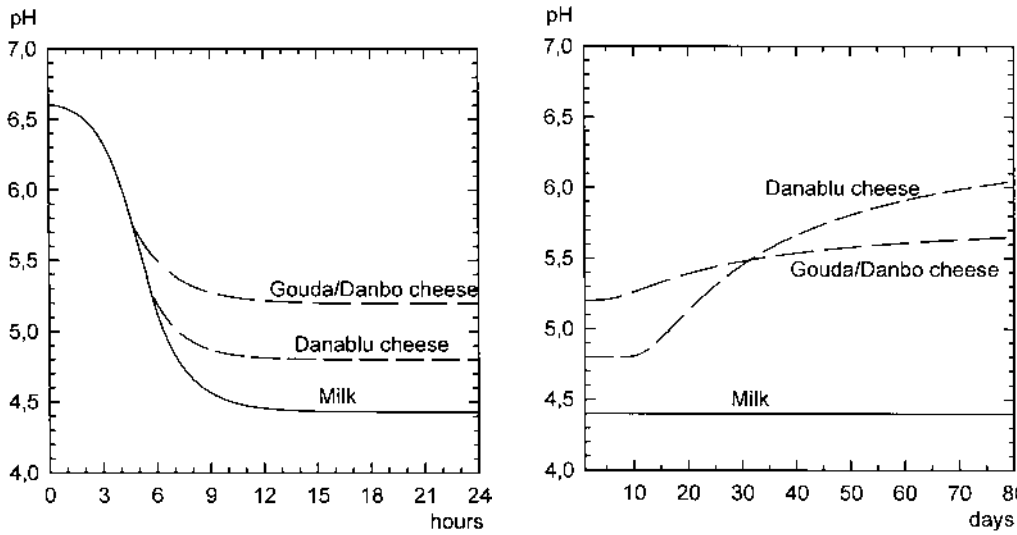

Figure 2 The course of acidification for a Gouda/Danbo cheese, for a blue-veined cheese (Danablu), and for milk, respectively, during the first 24 hr after inoculation with 1% mesophilic starter culture, all starting at 30°C (left). The graph to the right show pH changes during storage and ripening. The fall in pH during the first 24 hr is caused by the fermentation of lactose into lactic acid by the lactic acid bacteria. The rise during storage is caused by release of amino acids and ammonia by breakdown of proteins and by degradation of lactic acid. The bottom curve applies to milk inoculated with lactococci and which is protected from infection by yeasts and molds during storage. (From Ref. 1.)

mixture of whey and cheese grain in order to dilute the whey, thereby reducing the lactose concentration.

However, the pH minimum is not solely a function of the ratio of lactose to buffers. As long as the curd grains are dispersed in the whey, lactose can diffuse from the whey into the grains in replacement of that which has been fermented into lactic acid during stirring. (About 90% of the lactic bacteria are being concentrated in the curd grains, hence the lactose fermentation mainly takes place here.) Consequently, the total decrease in pH has to be looked upon as two phases: *phase one*, the decrease of pH in the curd grains until their final separation from the whey, and *phase two*, the decrease in pH in the cheese curd after it has been finally separated from the whey; in this phase, the decrease in pH is determined solely by the ratio of lactose to buffers.

An approximate calculation of pH minimum:

$$\text{pH minimum} = \text{pH}_1 - 0.8298 \times (\text{L/B}) \times (\text{pH}_1)^2 + 24.89 \times \text{pH}_1 \times (\text{L/B})^2$$

where pH_1 is pH at molding/start of pressing (when cheese grains are finally separated from the whey); L is % lactose hydrate of the pressed cheese, and B is buffering capacity = % protein of the pressed cheese $+ 19 \times$ % inorganic phosphate (5). For Cheddar cheese, the addition of salt to the milled curd, depending on the concentration of salt, may delay or stop the acidification before all lactose has been completely fermented (5). In the model it is assumed that all lactose is converted to lactic acid. Lactose is calculated here as hydrate, therefore one weight unit of lactose corresponds to one weight unit of lactic acid.

Calculation

$$L = \frac{Lm(100 - A(100 - Tw - h * Pw)/(100 - Tm - h * Pm)) * ((100 - Tc - h * Pc)/100)}{(100 - Tm - h * Pm) - A(100 - Tw - h * Pw)/100 + W},$$

where Lm is % lactose hydrate in milk, Tm and Pm are % total solids and % protein, respectively, in milk, Tw and Pw are % total solids and % protein in whey, and Tc and Pc are % total solids and % protein in cheese (24 hr). A is the amount of whey (% of milk by volume) removed during stirring, W is water (as % of milk by volume) added to the mixture of whey and curd grains during stirring. h [exclusion factor (2)] is amount of water bound to protein in such a way that it cannot function as solute for lactose. h can be set to 0.3 g H_2O per g protein (6).

F. Nonbacterial Acidification

In some countries, it is allowed for certain types of cheeses (e.g., quarg, cottage cheese, and mozzarella) to acidify the curd by phosphoric-, acetic-, or citric acid, or by glucono-delta-lactone, which slowly is converted to gluconic acid.

G. Other Microorganisms

1. *Propionibacteria*. For Emmental cheese and other types with large eyes, cultures of *Propionibacterium* sp. are added in order to initiate fermentation of lactic acid to propionic acid, acetic acid, and CO_2; the latter contributes to the formation of the large holes.
2. *Molds*. For blue-veined mold cheeses, spores of *Penicillium roqueforti* are added to the milk. For white mold cheeses, spores of *Penicillium camemberti* and other *Penicillium* species are added to the milk or onto the surface of the cheese.
3. *Secondary flora of lactic acid bacteria*. In semihard cheeses, the number of starter bacteria culminates at about 10^9 cells per gram after 1–2 days. Thereafter these bacteria gradually die out and autolyse. During the first month of ripening, 99% of the lactococci may die out, and simultaneously a secondary flora of lactobacilli develops and may grow to a number of 10^7–10^8 cells/g (7). These lactobacilli, mainly belonging to the group of facultatively heterofermentative lactobacilli (e.g., *Lactobacillus casei/paracasei*), develop spontaneously in the cheese and are found only in low numbers (e.g., 1–10 per milliliter, or less, in pasteurized cheese milk); they may originate from the flora in the cheese factory or they may be lactobacilli surviving pasteurization. Pediococci have also been found in the secondary flora. Although the presence of a secondary flora in cheese has been known for more than one hundred years, knowledge of their growth and their effects on the cheese is still relatively scarce. There are indications that the secondary flora may have positive effects on the quality of cheese: for example, by inhibition of detrimental bacteria such as heterofermentative lactobacilli (8) and *Clostridium tyrobutyricum* (9) and by the consumption of oxygen diffusing into the cheese (10).

H. Rennet

The primary effect of rennet is coagulation of the milk. Later, rennet enzymes play an important role in the hydrolysis of proteins during ripening. The coagulating activity of calf rennet, or standard rennet, is due to the enzyme chymosin. Traditionally, rennet is made

from the fourth stomach of calves, the abomasum, which is sliced and extracted in a weak acid salt solution. The extract is filtered and the pH adjusted to 5.5. Finally, 15–20% salt and benzoic acid is added. Milk-coagulating enzymes can also be extracted from stomachs from oxen or from other animals, by the fermentation of certain molds, or by microorganisms into which the gene for chymosin has been cloned.

I. The Enzymatic Coagulation Process

Coagulation takes place in two phases. First, a negatively charged part of the k-casein (one-third of the κ-casein molecule) is split off by hydrolysis (catalyzed by the rennet enzyme) of one peptide bond (no. 105, phenylalanin/no. 106, methionin) in κ-casein. The casein micelles thereby lose a part of that negative charge that otherwise prevents the micelles from coagulating. The casein (paracasein) is now insoluble in the presence of Ca^{2+}. In the second phase of renneting, the paracasein micelles aggregate by hydrophobic attraction between hydrophobic amino acid residues in the caseins. With 30 mL standard rennet per 100 L and at 30C, the first phase takes about 10 min and the second phase about 1 min. It then takes about 20 min before the coagulum is sufficiently firm for cutting.

J. Addition of Calcium Chloride

Addition of $CaCl_2$, 5–20 g per 100 L, can increase the rate of renneting partly because the addition of $CaCl_2$ gives a slight reduction in pH.

V. PRODUCTION OF CHEESE CURD

After the milk has formed a firm coagulum, the next steps (cutting, stirring, and heating/scalding) have the purpose of controlling the syneresis of the curd in order to achieve the appropriate level of moisture.

A. Cutting

The coagulum is cut in grains by means of knives or fine steel wires. The size of the grains influence the syneresis—the finer the cut, the lower the moisture content in the cheese. For soft cheese, the grain size is 15 mm or larger; for semihard cheese, 5 to 10 mm; and for hard cheese, 2 to 5 mm.

B. Stirring

After cutting, whey begins to be squeezed out of the grains and the syneresis begins. The mix of whey and grains is then stirred, at first gently, in order to enhance syneresis. The stirring is continued until the grains have reached the desired firmness. The stirring may take from one to two hours. The main factors for the syneresis are fat content of milk, size of the grains, pH, and temperature during stirring. Lower fat, smaller grains, lower pH, and higher temperature increase syneresis.

C. Heating/Scalding

Heating during stirring is not usual for soft and semisoft cheeses; however, for semihard cheese, heating to 34–38C is typical, for hard/semihard cheeses 37–40C, and for hard cheeses

50–56C (scalding). The heating/scalding can be done by heating the mixture of whey and grains by steam in the jacket of the cheese vat or by the addition of hot water to the whey. Addition of water reduces the lactose content of the curd and, consequently, the amount of lactic acid produced in the pressed cheese. Another effect of heating/scalding is that the lactic acid bacteria, and their acid production under stirring, will be inhibited because the temperatures applied are above their optimum.

If a higher heating temperature for Gouda/Danbo cheese is used, the starter bacteria will grow slower in the cheese grains, and the pH at the end of stirring/molding will consequently be higher. Although higher pH during stirring will in itself reduce syneresis, the higher heating temperature will increase syneresis. Experience shows that the net result will be lower moisture content in the cheese. A higher heating temperature will also cause a higher pH minimum of the cheese because the cheese grains will have higher pH at the end of stirring/at molding *and* because the pressed cheese has a lower moisture content, and consequently, a lower content of lactose. In summary: Higher heating temperatures for a semihard cheese such as Gouda/Danbo type will result in a more firm and a less acid cheese.

D. Final Treatment of the Cheese Curd

When the cheese grains have reached the desired firmness, they must be separated from the whey. The curd can then be put directly in molds or it can be given further treatment before final molding.

On the whole, a few different molding methods are used for the many varieties of cheese:

Curd grains are pressed under whey before molding (round-eyed cheeses).
Curd grains are pressed under whey before molding and boiled in whey (Halloumi cheese).
Grains are separated from the whey before molding (granular cheeses, soft cheeses).
Grains are separated from the whey and the curd is left a few hours for continued acidification (cheddaring) and then milled, salted, and molded (Cheddar, Stilton) or milled and then warmed and kneaded before molding (e.g., mozzarella).
After being concentrated by ultrafiltration and added rennet, etc., the cheese is cast directly in molds or in the package (e.g., UF-Feta).
Sour milk cheese: After acidification of the milk to about pH 4.6, the whey is separated from the precipitated curd, which can then be packed for consumption (Tvorog, Quarg, Skyr) or formed into small cheeses and ripened (Olmützer quargeln, Harzer käse, Pult ost).
Grains of acid-precipitated coagulum, gently cut and stirred and heated, are separated from the whey and the loose grains are packed for sale (cottage cheese).

The following describes in more detail some of the above-listed different principles for handling the curd.

1. Round-Eyed Cheeses

The curd grains are collected under whey, avoiding mixing with air; lightly pressed and cut in pieces, one for each cheese; placed in molds and finally pressed for 1 to 2 hr or more. The molds are equipped with cloths or perforated for drainage of whey and for formation of a

closed rind on the cheese. This method results in a closed texture with only microscopic bubbles of air. The closed texture is an important factor for obtaining the wanted structure of round-eyed cheeses. The starter cultures used for semihard round-eyed cheeses (e.g., Gouda/Danbo) contain bacteria that in 1 to 2 weeks can ferment the citric acid, thereby producing CO_2.

Initially CO_2 is dissolved in the cheese, but eventually the combined pressure of CO_2 and that of the N_2 dissolved in the cheese begins to expand the largest of the microscopic air bubbles. If the velocity of the formation of CO_2 is suitable, the gas will expand only a few of the largest air bubbles because of the following physical law:

Pressure in a bubble = surface tension/radius of the bubble

which states that less gas pressure is required for enlarging a large hole than a small one (11). The same applies for large-eyed cheeses (e.g., Emmental and Jarlsberg), where the eyes are expanded by CO_2 produced by propionic acid bacteria by fermentation of lactic acid.

2. Halloumi Cheese

After stirring, the grains are pressed under whey, then cut in 300 g pieces, put into boiling whey, and kept here at about 90°C for 30 min. After cooling, the Halloumi cheese is stored in brine.

3. Granular Cheeses, Soft Cheeses

The whey is drained from the curd grains, which then are scooped into molds. If the grains are relatively firm, as in semihard cheeses (e.g., Tilsiter), air will be mixed in between the grains, which the cannot fuse completely; therefore, a large number of tiny air pockets will be incorporated, giving a granular texture. When CO_2 is formed during ripening, the gas enlarges the air pockets, producing irregularly shaped holes.

In blue-veined cheeses (e.g., Roquefort and Danablu), the air pockets, enlarged by CO_2 from bacteria and yeasts, make up the spaces in which *Penicillium roqueforti* can grow, provided atmospheric oxygen is given access via openings in the cheese. If the curd grains are large and soft when put in molds, as for Camembert and Brie, the texture of the cheese can be without mechanical openings between the grains. Production of CO_2 may produce holes, but most of the CO_2 will diffuse out of small-sized cheeses. Soft cheeses are usually pressed only by their own weight.

4. Cheddar and "Pasta Filata" Cheese—Provolone, Mozzarella, and Kashkaval

The curd grains are separated from the whey, and the curd is left for a few hours for continued acidification (cheddaring), until a pH value of about 5.1 to 5.3 is reached. Then the curd is milled into chips.

For Cheddar chips, is added salt (2–2 ½%). Some of the whey, which has dissolved about 25% of the added salt, is squeezed out of the chips; the remaining 75% of the salt will be absorbed in the cheese. Finally, the curd is hooped, pressed, wrapped in plastic film, and packed in cartons and placed in storage for ripening.

In the production of provolone, mozzarella, and Kashkaval, the chips of milled, acidified curd are conveyed or shoveled into a container filled with hot water or brine at 75–85°C, and the curd is kneaded until it is smooth and elastic. The curd then is formed in molds and cooled.

VI. SALTING OF CHEESE

Salt is important for conservation of cheese, for a balanced, pleasant taste, and for consistency. For many cheeses, salting also plays a role in reduction of the moisture content. For most semihard and semisoft cheeses, 1.5–2% NaCl is suitable. For Emmental and other large-eyed cheeses, the salt content is lower—0.8–1.4%, in order to facilitate the growth of propionic acid bacteria. For blue-veined cheese, 3–3.5% NaCl is suitable.

The salting can be achieved either by adding salt to the curd before molding or by diffusion of salt into the cheese after molding, by immersion in brine, or by dry salting. The lactic acid bacteria are sensitive to salt and will be severely inhibited if the cheese is salted before acidification has been finished.

A. Salting Before Molding

For Cheddar cheese and Stilton, the curd is allowed to continue acidification until about pH 5.2 during the cheddaring process. Then the curd is milled, salted, and molded.

B. Salting After Molding

For most other cheeses made without a cheddaring process, the curd is put in molds and pressed shortly after the end of stirring. At that point the pH typically will be about 6.2–6.4, and if more than a small amount of salt is added, the growth of starter bacteria and the acidification would be severely inhibited; the formed cheese therefore has to be salted by absorption of salt from dry salt or from brine.

1. Dry Salting

For dry salting, the surface of the cheese is rubbed with dry salt or a salt paste.

2. Brine Salting

For salting in brine, cheeses are immersed in brine (20–22% NaCl) at 10–15°C. Salt is taken up by the cheese while at the same time some moisture is forced out. The loss of moisture, which is higher than the uptake of salt, gives the cheese a firm rind. The uptake of salt and loss of moisture depends on the geometrical dimensions of the cheese, its moisture content, its fat content, and the concentration of the brine.

Some examples for salting duration in brine with 20–22% salt:

Grana cheese, high cylinder, 45 kg, 1.7% NaCl: 3 weeks
Emmental cheese, flat cylinder, 100 kg, 0.8–1% salt: 3 days
Semihard cheeses (Gouda/Danbo, etc.), 2–12 kg, 1.6–2.2% NaCl: 1–3 days
Soft cheeses (Camembert, Brie, etc.), 0.1–1 kg, 1.5–2.5% NaCl: 0.5–3 hr

The uniform diffusion of the absorbed salt thoughout the cheese may take up to several weeks, depending on the weight, moisture content, and dimensions of the cheese.

VII. RIPENING OF CHEESE

Although cheeses like cottage cheese and quarg are consumed fresh, most cheeses require a ripening period (from a few weeks up to several years) for development of characteristic

flavor, consistency, and appearance. The changes during ripening are brought about by enzymes and microorganisms. The effects of the enzymes and the microorganisms depend on pH and temperature, on the content of moisture and of salt, and on the surface treatment.

Examples of regimens for storage for ripening:

Emmental: 2 weeks at 12°C; 3–6 weeks at 20–24°C; then at 8–10°C
Cheddar: 4–12 months at 6–10°C
Semihard cheeses: Gouda/Danbo, etc.: 2–4 weeks at 12–18°C; then at 6–10°C
Blue-veined cheese: 3–5 weeks at 8–12°C; 1–3 months at 2–6°C
White mold cheese: 1–2 weeks at 12–15°C; 1–2 weeks at 6–10°C; 2–6 weeks at 2–5°C

A. Fermentation of Lactose and Citric Acid

The fermentation of lactose to lactic acid by lactic acid bacteria, and the fermentation of citrate by citrate using bacteria in DL-starters, is described in the sections above. In cheeses such as Emmental and other large-eyed cheeses, lactic acid is further fermented by propionic acid bacteria to propionic acid, acetic acid, and CO_2.

B. Proteolysis

The caseins are gradually hydrolyzed by rennet proteinase enzymes and by plasmin, an indigenous milk protease, which yields a number of large polypeptides; the latter do not influence the flavor of the cheese, but some hydrophobic peptides may be astringent or may have a bitter taste. These first steps of the proteolysis are important for changing the structure of semihard cheeses from a rubberlike to a sliceable consistency.

Some of the peptide bonds in the polypeptides can be further hydrolyzed by enzymes from the lactic acid bacteria to yield smaller peptides and free amino acids, contributing to the basic taste of cheese. Amino acids may be further converted to smaller molecules—ammonia, organic acids, amines, esters, low molecular sulfur compounds, etc.—contributing to the aroma of ripened cheese.

1. Extent and Depth of Proteolysis

The velocity of the breakdown of proteins and the amounts of resulting compounds produced during ripening are very different for the various kinds of cheese and depend on a range of factors: type and amount of rennet, type of starter bacteria, level of moisture, pH of the cheese, salt%, and storage temperature.

The progression of proteolysis can be monitored by electrophoresis, by HPLC, and by separation of the casein and its breakdown products into groups according to their solubility. In the classic ripening analysis, the extent of proteolysis is defined as the amount of total nitrogen that is soluble at pH 4.4 (12).

The depth of the ripening is defined as the amount of the nitrogen present as amino acids and low molecular nitrogen compounds. The amount of free amino acids can be estimated by nitrogen determination in the filtrate after precipitation with phospho-tungstic acid (13), by a titration with formaldehyde (14), or by other methods (15).

Fig. 3 shows curves for soluble nitrogen production during ripening for blue-veined Danablu cheese, for semihard Danbo cheese with moderate smear ripening, for Cheddar cheese, and for Feta cheese. For the Danbo, curves are also given for amino nitrogen (determined by phospho-tungstic acid or by formol titration) and for NH_3 nitrogen [based on data from (5) and (16)].

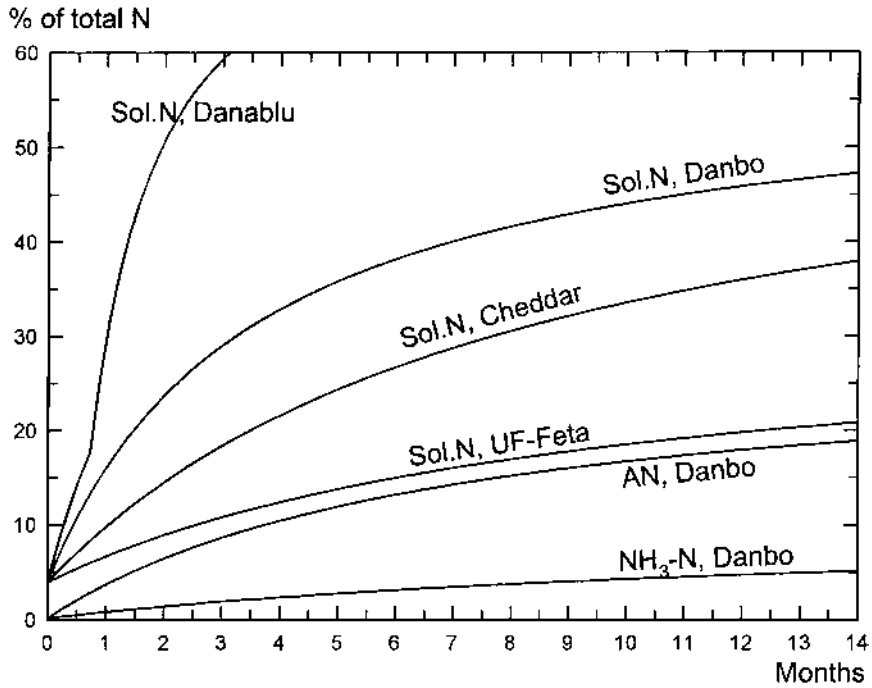

Figure 3 Typical development of soluble nitrogen at pH 4.4 during ripening as percent of total nitrogen for Danablu cheese, for semihard Danbo cheeses and similar Danish cheeses with moderate smear ripening, for Cheddar cheese, and for feta cheese (made by ultrafiltration). For Danbo and similar Danish cheeses, curves are also shown for typical development for amino nitrogen (determined by precipitation with phosphotungstic acid or by formol titration) and for NH_3 nitrogen during ripening. (From Refs. 5 and 17.)

2. Surface-Ripening

Aerobic microorganisms may develop on the surface of cheese. If the surface is moist, bacteria and yeasts may form a red-brownish smear, in which protein and fat is catabolized, producing fatty acids, amino acid breakdown products such as ammonia, and various low molecular sulfur compounds; these compounds diffuse into the cheese and impart a spicy flavor, the intensity of which depends on the growth of the surface flora, the dimensions of the cheese, its moisture content, and the temperature and duration of surface ripening.

3. Feta and Similar Cheese

Feta and similar cheeses are acidified to a low pH, about 4.4–4.6, and kept in brine. The enzymes of rennet retain some activity; the peptidases of the lactic acid bacteria, however, are practically inactive at this low pH. Hence, proteolysis in Feta is rather limited, the amounts of free amino acids and ammonia produced are low, and there is no increase in pH during ripening.

4. White Mold Cheeses

Like Feta, fresh white mold cheeses are low in pH, about 4.5–4.7. The ripening of a Camembert cheese is dependent on the growth of white mold on its surface, which consumes

lactic acid and produces ammonia from proteins. The pH of the rind soon rises to pH greater than 7, and a pH gradient is created from the surface to the center (17). The pH increases gradually throughout the cheese, thereby activating peptidases of the lactic acid bacteria, releasing amino acids from the peptides. The ammonia and other metabolic products from the mold diffuse into the cheese and contribute significantly to the flavour.

5. Blue-Veined Mold Cheeses

The pH of fresh blue-veined cheeses is rather low, about 4.6–4.8, so a complete ripening process is dependent on the growth of mold, analogous to that described above for white mold cheeses. *Penicillium roqueforti* is able to grow at rather low oxygen tension, hence it can develop in openings and caves inside the cheese if there is oxygen access via perforations of the cheese. The molds in blue-veined cheeses produce highly active protein (and fat)-degrading enzymes.

C. Lipolysis

By lipase enzymes the triglycerides of milk fat can be hydrolyzed into free fatty acids and glycerol. The short chain fatty acids have a sharp, pungent flavor, which although undesirable in milk and butter may contribute to a balanced flavor in some ripened cheese because of these fatty acids and other metabolites such as metyl ketones and esters. In some cheeses, lactones to produced from hydroxy acids of the fat—may also contribute to flavor.

1. Lipolytic Activity in Cheese

a. Milk Lipase. The indigeneous lipase of milk can produce a significant lipolysis in cheese made of raw milk (Table 6). Pasteurization of the milk at 72°C for 15 sec inactivates about 95% of the milk lipase.

b. Rennet and Starter Bacteria. Standard rennet does not contain lipase and the starter cultures of lactic acid bacteria do not produce enzymes that can liberate fatty acids from milk triglycerides.

Consequently there will be only slight lipolytic activity in cheese made of good-quality pasteurized milk.

Table 6 Examples of Typical Acid Degree Values for Cheese Fat

Cheese	Acid degree[a] (BDI)
Nonhydrolyzed milk fat	0.5–0.8
Flavor threshold value for milk	1.2
Semihard cheese from raw milk, ripened 6 months	3–5
Semihard cheese, made of pasteurized milk of good quality, ripened 6 months	1–2
Semihard cheese, made of pasteurized milk with 500,000 psychrotropic bacteria per mL before pasteurization, ripened 6 months	3–5
Blue-veined cheese (Mycella) made of raw milk, ripened 3 months	10–20
Blue-veined cheese (Danablu) made of raw homogenized milk, ripened 3 months	30–50

[a] mL 1 N alkali per 100 g fat.
Source: Ref. 16.

c. Added Enzyme Preparations. Traditional rennet paste, made by macerating stomachs plus pregastric region of lambs or kid goats, contains pregastric lipases with high specificity for the liberation of short chain fatty acid, which in cheese can contribute to a piquant flavor (e.g., Provolone and Feta) (18).

d. Lipases from Psychrotropic Bacteria. In raw milk, psychrotropic, gram-negative bacteria may produce lipases (and proteases). These bacteria are killed by pasteurization, but their enzymes may persist (19). If the milk is high in psychotropic bacteria before pasteurization, thermo-resistant bacterial lipases can split fat during ripening. Concomitantly, an unclean flavor may develop, due to thermoduric protein degrading enzymes from the psychrotropic bacteria.

e. Lipases from Surface Microflora. Bacteria and yeasts in the smear on surface-ripened cheesescan hydrolyze fat on the surface, and the liberated fatty acids can diffuse into the cheese (20) and contribute to the flavor, in combination with ammonia and other low-molecular-weight compounds from degraded proteins of the surface smear.

f. Mold Lipases. The molds of blue-veined and white-mold cheeses produce very active lipases. However, in Camembert, for example, the contribution of free fatty acids to the flavor is reduced because of the dissociation of the acids at the high final pH.

2. The Influence of Homogenization on Lipolysis

Homogenization increases the effect of lipase dramatically. If raw milk is homogenized (e.g., for blue-veined cheese), then significant lipolysis, catalyzed by the milk lipase, will occur quickly in the cheese vat. The combined effect of milk lipase and the mold lipases during ripening is also increased by homogenization of the milk (21).

3. Extent of Lipolysis

The hydrolysis of fat can be measured by titration of fat extracted from the cheese—for example, by boiling with BDI-reagent after adjustment of pH to 4 (21). This method recovers about 70% of the free fatty acid. Table 6 give some examples of acid degree of fat from various cheeses.

D. Finishing the Cheese

Hard and semihard cheeses, after a stable rind has been formed, are often given a layer of cheese wax in order to prevent further loss of moisture. For semihard cheeses with surface ripening, the smear should be removed after 2 to 3 weeks, and the cheese dried carefully, before waxing can be applied. Mold cheeses, soft cheeses, and formed, ripened sour milk cheeses usually are packed in aluminum foil. Feta-type cheeses and Halloumi, kept in brine in containers, are sold directly from the container or packed in plastic bags with brine.

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13
Traditional Greek Feta

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I. INTRODUCTION

Cheese has always been an important component of the Greek diet. Greece has the world's second highest cheese consumption: 24.56 kg cheese per capita per year (1). In 1998 the cheese consumption in Greece was 269,350 metric tons; 49.5% of this was Feta.

References to cheese production go back to the 8th century B.C. The cheesemaking technology described in Homer's *Odyssey* is similar to the technology used until recently by Greek shepherds to make Feta cheese from the milk of their sheep and goats. Other ancient Greek authors also mention cheese made from goat's or sheep's milk as common Greek food. Aristotle (384–322 B.C.) goes a little further and says that cheese is composed of water, fat, and *tyrine*, a term used in Greece up to the early 20th century to designate casein (in Greek, *tyri* = cheese).

Feta cheese is a white, semihard cheese made from sheep's milk (or mixtures of sheep's and goat's milk), which is ripened and stored in brine. Traditionally, the cheese was packed in blocks in wooden barrels; the shape of the cheese blocks looked like watermelon slices. The name Feta, which means 'slice' in Greek, has probably come from this original shape of the cheese or, perhaps, from a property of the cheese that enables it to be sliced without falling apart (2). Besides Feta, many named varieties of other pickled cheeses are traditionally produced in southeast Europe and the Middle East. The most well known among them are Bjalo Salamureno Sirene (Bulgaria), Beyaz Peynir (Turkey), Beli Sir u Krišķama (Yugoslavia), Teleme or Telemea (Greece, Romania), and Touloumisio (Greece). The majority are made from sheep's milk: the sheep farming is very important in countries where climatic conditions are not favorable for cattle raising.

Feta, the most well known variety of this group of cheeses, is very much appreciated in Greece, and, in most cases, is synonymous with cheese. According to the data from the Hellenic Ministry of Agriculture, the annual production of Feta in Greece is more than 130,000 metric tons, most of which is consumed within the country. However, because of its unique sensory properties and the fact that large Greek ethnic groups live in many countries around the world, small quantities of Feta are exported, mainly to the United States. Over time, the name Feta has acquired an important trade value and is now being used to designate all pickled cheeses having similarities to Feta but made from cow's milk,

even if made using a completely different technology. These cheeses cannot duplicate the typical flavor of traditional Feta cheese, however, and do not fulfill consumers' expectations. In 1996, the European Commission recognized that the designation "Feta" qualifies for a Protected Designation of Origin (PDO) according to the Regulation 2081/92 (3). The designation was awarded only to the particular pickled cheese produced on the mainland of Greece and on the island of Lesvos from sheep's milk or mixtures of sheep's and goat's milk.

Until recently, Feta was made in small cheese plants using the milk produced in the region. The sensory characteristics of the product differed from place to place. During the past decades, large dairy industries, able to collect large quantities of sheep's and goat's milk, have started to produce Feta cheese; now, the milk is pasteurized and the process is mechanized. This has led to stabilization of high quality and sensory properties throughout the year. Nevertheless, the special sensory characteristics of the cheeses made in farmhouses or in small mountain plants using the local milk are still appreciated by connoisseurs.

II. MANUFACTURE OF FETA CHEESE

A. Milk

The most suitable milk for Feta cheese is sheep's milk but a mixture of sheep's and goat's milk may be used. However, the percentage of goat's milk in the mixture is not allowed to exceed 30% (4). Goat's milk alone can also be used for pickled cheese manufacture, but the cheese, although appreciated by consumers preferring a stronger taste, is not allowed to be called Feta. The production of sheep's and goat's milk is seasonal (December/January to June/July); therefore, the activities of Feta cheese plants are restricted to only 6–8 months.

There is a significant variation in fat content during lactation, with lower values, at least for sheep's milk, corresponding to the beginning of the warm period. Protein in sheep's milk increases regularly as lactation advances (5). Thus, the fat content of artisanal cheeses usually depends on the production time. In contrast, in modern cheese plants the milk is standardized to a casein-to-fat ratio of 0.7–0.8; the fat content of the cheese is therefore more stable. For public health reasons, the milk is pasteurized and cooled to 34°C before the addition of starter culture. However, in small cheese plants or farms, the milk usually receives a thermal treatment lower than necessary for pasteurization conditions. In this case, part of the indigenous flora of the milk may survive, influencing the course of ripening and the release of flavor compounds.

B. Starters and Calcium Chloride

Starter culture is usually a combination of lactic acid bacteria at a ratio of lactococci to lactobacilli of 1:3 (e.g., *Lactococcus lactis* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). In small cheese plants, yogurt is sometimes used instead of pure cultures; in this case, acid development is slow (2). Culture is added to cheese milk to a level of 0.5 to 1% (v/v) and incubated for about 30 min. Calcium chloride up to 20 g per 100 kg milk may also be added, although this addition is not necessary when unpasteurized sheep's milk is used. Indeed, sheep's milk is rich in calcium, a fact related to the high casein content (6,7). Concentrations as high as 172 to 209 mg Ca per 100 g are reported (8).

C. Coagulation

Coagulation by rennet is performed at 32–34°C for 45–50 min. Sheep's milk is very sensitive to rennet and, because of the higher β/α_s-casein ratio, coagulation proceeds faster than with cow's milk (9,10). Alternatively, less rennet is required to obtain the same coagulation time as with cow's milk (11). The use of homemade rennet, made from the abomasa of unweaned lambs and kids (12), was very common when the majority of Feta cheese was manufactured at small plants in mountainous and semi-mountainous regions. Nowadays, the modern large and medium-sized cheese plants use commercial calf rennet.

D. Draining

After coagulation, the curd is cut crossways in cubes of 2–3 cm, left for about 10 min for partial exudation of the whey, and transferred into perforated molds. Molds are cylindrical when the cheese is to be packed in barrels and rectangular when intended for packing in tin cans. As layers of curd are piled in the mold, small mechanical openings are formed. These openings, full of whey, are typical of Feta cheese structure.

The curd is left to drain at room temperature without pressing, until it is firm enough to remove the molds. Ovine curd drains less than bovine because of its higher total solids, casein, and fat content, and its higher firmness. In contrast, caprine curd is usually drier than bovine because goat's milk contains less casein—especially α_{s1}-casein—and calcium than sheep's milk (8,9). Feta cheese producers profit from this behavioral difference and usually mix 10–30% goat's milk with sheep's cheese-milk because the curd remains firm and drains better (8). The time needed for draining depends on the temperature. Environmental temperatures of 14–16°C are common but higher temperatures are not rare during late spring. Composition of the starter culture also affects draining because the rate of acidity development is crucial for the quality of Feta cheese. The gradual cooling of the curd from 30 to 16°C allows the starter microorganisms the time and the environmental conditions to develop acidity and enhance the draining process. Mesophilic starters are found to lower the pH faster than thermophilic ones and to shorten the draining time; however, a combination of mesophilic and thermophilic strains are more suitable (13). A pH drop to 5.0–5.2 in 8 hr and to 4.8–5.0 in 18–24 hr is considered necessary for proper drainage and ripening (2). Table 1 shows the typical pH evolution during the manufacture of Feta cheese.

Table 1 Typical pH Changes During Feta Cheese Manufacturing

		pH (mean values)	
	Time from start (hours)	A	B
Milk	0	6.59	
Milk, incubated with starters[a] (before renneting)	0.5	6.39	
Curd, during dry salting	2		6.09
	4		5.72
Curd, at the end of draining	8	5.12	5.16
Curd, at the next morning	20–21	4.70	4.91

[a] A: *Lactococcus lactis* and *Lactobacillus delbrueckii* var. *bulgaricus* (1:3) (14); B: *Lactococcus lactis* (2 strains) and *Lactobacillus delbrueckii* var. *bulgaricus* (2:2) (13)

Source: Data adapted from Refs. 13 and 14.

E. Salting

Usually, after 2–3 hr the molds are inverted and the curd is usually left for another 2–3 hr to complete draining. The molds are then removed and the pieces of curd transferred on a salting table spread with granular salt. The pieces are placed side by side to retain their shape, and their exposed surfaces are sprinkled with salt the size of rice grains. The salt, dissolved in the exuded whey, slowly penetrates into the cheese without causing the formation of a dry rind. After about 12 hr the cheese pieces are turned and two other surfaces salted. This process is repeated four more times at 12-hr intervals until each surface is salted twice and the cheese contains about 3% salt. During draining and salting, the pH of the curd continues decreasing to about 4.8.

F. Ripening

Following salting, the cheeses remain on the table for two more days depending on the ambient temperature. They are inverted at regular time intervals. During this period, a slimy layer is formed on their surfaces due to the growth of bacteria and yeasts (12); the composition of this surface flora depends on the environment of the cheese plant. Gradual dry salting, low pH, and slime formation are essential for the development of the typical flavor of Feta cheese; it is found that the proteolytic and lipolytic activity of the surface microflora is significant during the first 15–20 days of ripening and contributes to the release of many peptides, amino acids, and fatty acids, precursors of most volatile flavor compounds (2).

In modern dairy industries, molding, drainage, salting and preripening are mechanically performed. The curd is transferred into the molds by gravity. Salting takes place in open tin cans, which can be automatically inverted when needed. Also, salt is of high purity and, therefore, subsequent washing of the cheese pieces is avoided.

G. Packaging and Storage

Feta cheese is packed in wooden barrels or in tin cans. Barrels were the traditional containers for Feta, and when stored in barrels, the cheese develops a stronger and spicier flavor, much appreciated by consumers. However, handling a filled barrel (weighing about 50 kg) is difficult, so today Feta cheese is packaged mostly in tin cans, which weigh 16–17 kg, making their transportation easier.

Before packaging, the surface of the cheese pieces is carefully cleaned with water or brine. In the container, each layer is usually covered by a piece of parchment paper, before the next layer is added. Finally, 1.0–1.5 kg of 6–7% brine is added in order to cover the cheese. Cheese pieces must be tightly packed, allowing little space for the brine. If the volume of brine is larger than necessary, many low-molecular-weight compounds diffuse from cheese into the brine (see Sec. V.A.1). From time to time, it is advisable to let the fermentation gases escape and to fill the container with brine if the level fails to cover the cheese. Salt concentration profiles of brined cheese are temperature dependent; salt diffusion varies with temperature according to the Arrhenius equation (15).

The barrels or tins are kept at 16–18°C until the pH of the cheese reaches 4.4–4.6 and the moisture drops to less than 56%. They are then transferred to cold rooms (4–5°C) to complete ripening. Marketing of Feta cheese is permitted only after 2 months postmanufacture (4). Until sold to the consumer, the cheese must be kept in brine. If uncovered by brine, the surface becomes dry, changes color, and permits the growth of yeasts and molds. A good-quality Feta cheese may be stored in brine for up to 12 months at 2–4°C.

After ripening, Feta cheese slices may also be individually packed in plastic bags under vacuum for retail marketing. Once this packaging is opened, however, the cheese has to be consumed within a few days. An alternative consumer packaging is the placing of the cheese slice in a small plastic container filled with brine, permitting longer storage at home (at 4–5°C).

H. Yield

Since the average dry matter of sheep's milk is 18–20% (8), a yield of about 25% is expected (12,16,17). This means that about 4 kg of milk is needed to produce 1 kg of cheese. The yield varies with season (a little higher in winter than in spring) and with the percentage of goat's milk mixed with the sheep's milk.

III. GROSS COMPOSITION AND SENSORY PROPERTIES

A. Gross Composition

The gross composition of Feta cheese is shown in Table 2. The compositional provisions of the Greek Food Code for Feta are as follows: maximum moisture 56% and minimum fat in dry matter (DM) 43%.

Few rheological analyses have been conducted on Feta cheese, as Feta is a difficult material that easily breaks into pieces when compressed. From the data available, hardness seems to vary from 4 to 7 kg, fracture stress from 1.8 to 2.4 kg, and compression to fraction from 14 to 22% (13,19).

B. Sensory Properties

Feta has a short, firm, and smooth texture, a moist surface without rind, and it is sliceable. Mechanical openings distributed over the cheese are normal, but the presence of small round holes is regarded as a defect, indicating anomalous fermentations. The color of Feta is bright white because sheep and goats transfer very few carotenoids to their milk.

The taste of Feta cheese is slightly acid and salty and its flavor rich and mildly rancid due to the relatively high level of low- and medium-chain free fatty acids. Feta made with mixtures of sheep's and goat's milk has, in general, a stronger but still pleasant flavor appreciated by some consumers. The sensory characteristics of traditionally produced Feta in small dairies vary from place to place because of variations in the technology parameters applied, including the starter culture used and the indigenous microflora of the milk and of the environment in each plant. Additionally, the vegetation in local pastures is different in places having a different microclimate, and this imparts different flavors and aromas from

Table 2 Gross Composition of Feta Cheese

	Average value
Moisture %	54.6
Fat %DM	49.1
Protein % (N × 6.38)	17.1
Salt (% of the aqueous phase)	5.3
pH	4.5

Source: Data compiled from Refs. 2, 12, 18–20.

the milk to the cheese. This variability is expected by the consumer. However, large cheese plants, with milk collected from a larger region, pasteurize the milk and, because of a better control of the technological parameters, produce a more standardized taste and flavor.

IV. MICROBIOLOGY OF FETA CHEESE

Mesophilic lactic acid bacteria constitute the main flora of Feta cheese (21,22). Their number significantly increases during the first 15-day period in the warm room (at about 16°C) and remains high throughout the ripening time (about 8 log cfu/mL). This explains the high proteolytic activity observed at the beginning of the ripening period.

Research has showed that nonstarter lactic acid bacteria (NSLAB) constitute a large part of this flora; they originate from milk (indigenous milk flora) and the environment of the cheese plant (21). Most species of NSLAB isolated from Feta are lactobacilli. They have an important role in the ripening process and contribute to the development of the characteristic flavor of the product due to the environment of this type of cheese, which favors their development and activity (21). *Lactobacillus plantarum* is the dominant species, representing about 50% of the isolates. Many different strains of this microorganism, isolated from Feta cheese, have been characterized as to their enzyme and plasmid profiles, acidifying and proteolytic abilities, and other characteristics; a marked genotypic variability is observed but phenotypic differences are small (23). NSLAB are also the dominant group of bacteria in the brine; *Lactobacillus paracasei* subsp. *paracasei* and *Lactobacillus plantarum* are the principal species identified (24).

Pediococci and enterococci are also present, but in lower numbers (21). However, investigations showed that some strains of *Enterococcus durans* (21) or *E. faecium* (25) could improve the sensory properties of Feta cheese if used as adjunct cultures in combination with mesophilic starters (e.g., *Lactococcus lactis* subsp. *lactis*, *Lactobacillus casei*, *Leuconostoc mesenteroides* subsp. *cremoris*). Micrococci are also found, mainly as part of the surface flora: staphylococci (not *S. aureus*), micrococci and coryneforms are the main species (N. Tzanetakis, personal communication, 1995).

Salt-resistant yeasts grow at high numbers (6–8 log cfu/mL) on the surface of Feta during dry salting but their number decreases with ripening time (26). Among the species that have been isolated, *Saccharomyces cerevisiae* was predominant (47.9% of the isolates) followed by *Debaryomyces hansenii* and *Pichia farinosa* (30.9% and 11.2%, respectively). Although they seem to be of minor significance for the ripening process (25,27), they do exhibit some aminopeptidase and esterase activity (26).

V. BIOCHEMISTRY OF FETA CHEESE RIPENING

Ripening is a delicately balanced process of controlled enzymatic breakdown (28). Proteolysis, lipolysis, and glycolysis lead to flavor compounds responsible for the sensory properties of the cheese. There is little data for glycolysis in Feta cheese in the literature, but lipolysis and, especially, proteolysis have been the object of many studies.

A. Proteolysis

1. General Observations

It is generally accepted that proteolysis is a major event during cheese ripening. Casein breakdown involves the action of many enzymes from various sources; among them, in-

digenous milk proteinase (plasmin), chymosin and other milk-clotting enzymes, proteinases and peptidases from the starter microorganisms and NSLAB, and enzymes from adjunct cultures, yeasts, and molds.

The extent of proteolysis in Feta cheese is, in general, lower than in most hard and semihard cheese varieties. During the 2-month minimum ripening time for Feta [according to the Greek Food Code (4)], the water-soluble nitrogen, expressed as % of the total N (WSN%TN) ranges from 12 to 20 (13,16,20,29); this depends somewhat on the residual rennet, the starter and nonstarter flora, and the time-temperature interaction during ripening. However, the WSN%TN, a parameter generally accepted as a "ripening index," does not reflect the products of casein hydrolysis. The water extract of a 3-day-old Feta also includes milk serum proteins, mainly β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). These proteins elute late from reverse-phase high performance liquid chromatography (RP-HPLC) columns and form large peaks (about the 25% of the total area) at the end of the chromatogram (30,31).

When the cheese is placed in brine, another process starts that also influences the N content of the water extract: the diffusion of cheese constituents into the brine (and, eventually, of salt into the cheese) until equilibrium is reached. Along the storage period, small and medium-sized nitrogenous compounds move into the brine. The solubility of casein breakdown products in 5% brine has been reported (32). Milk serum proteins, being also water soluble, follow in the same way. It was found that in chromatograms produced by RP-HPLC, the area corresponding to α-La and β-Lg decreases by about 40% (31). Therefore, it was proposed to substitute for WSN%TN as a ripening index another parameter, more closely related to the release of casein breakdown products. A colorimetric procedure based on the reaction of 2,4,6-trinitrobenzenesulfonic acid (TNBS) with free amino groups was found to give useful information on the degree of ripening of Feta cheese (33). In mature Feta samples, the correlation coefficient between WSN%TN and absorbance at 420 nm (A_{420}) was higher than in fresh samples, as the contribution of the whey proteins to the WSN was reduced (29).

2. Casein and Large Peptides

Proteolysis in Feta cheese is most intense during the first 15-day period, when the cheese is kept at a relatively high temperature (about 16°C). All soluble nitrogenous fractions show a significant increase at this time. Later, when the cheese is transferred to a refrigerator, the rate of casein and peptide degradation decreases (Table 3).

Table 3 Proteolysis Parameters During Feta Cheese Ripening

	Age (days)						
	1	3	15	30	60	90	120
Residual α_{s1}-CN	100	92.5	53.6	49.4	41.2	39.2	35.4
Residual β-CN	100	96.1	93.2	88.6	84.7	79.8	73.6
WSN%TN	6.2	11.2	14.9	15.2	16.1	18.1	19.0
TCA-SN%TN	3.0	5.2	10.8	12.1	12.5	14.1	15.8
FAA (mg/kg)	546	1016	2430	2498	2922	3073	3978

CN = casein; WSN%TN = water-soluble N% total N; TCA-SN%TN = 12% (w/v) trichloroacetic acid-soluble N% total N; FAA = free amino acids.
Source: Data compiled from Refs. 14, 16, 20, and 31.

Electrophoretograms of Feta on urea polyacrylamide gels (Fig. 1) reveal the degradation of casein during ripening. The breakdown of α_{s1}-casein starts very early. Many zones with an electrophoretic mobility higher than α_{s1}-casein are present in the 4-day sample; of these, the zones corresponding to the peptides produced by the action of chymosin (e.g., α_{s1}-I-casein) are the most intense. Hydrolysis of β-casein starts later, generating peptides with lower electrophoretic mobility than the parent protein (γ-caseins region). It is worth noticing that the intensity of all zones decreases during ripening, as proteins and large peptides are hydrolyzed into smaller fragments not able to be fixed on the gel. Only the production of the peptides with slow (γ-caseins region) and intermediate mobility (zone X of unknown identity) increases with time.

The results of many investigations (14,16,20,31) have showed that rennet plays an important role in Feta cheese ripening. From investigations (34,35) it appears that the extended α_{s1}-casein hydrolysis during early ripening is due to the residual rennet; because of the low pH and high moisture of the Feta curd, residual rennet is higher than for other cheese varieties (36–38). Additionally, the environmental conditions favor chymosin activity. Although chymosin is active on both α_{s1}- and β-casein, β-casein degradation is strongly retarded by the presence of salt (39). On the other hand, the activity of plasmin is relatively low, due to the low pH and the high NaCl content. The role of plasmin is mainly important in the ripening of cheeses in which rennet enzymes have been destroyed by high cooking temperature. This is not the case, however, with Feta. As a result, α_{s1}-casein is the main substrate of the proteolytic activity; 40–50% is hydrolyzed in 15–20 days (14,20), whereas the percentage of hydrolyzed β-casein is less than 10%. The later breakdown of

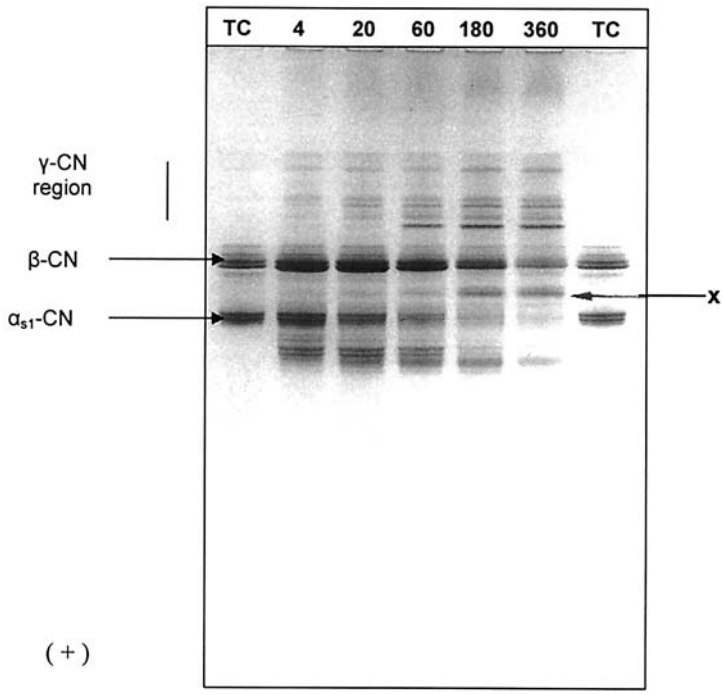

Figure 1 Electrophoretogram of Feta cheese at various ages (4, 20, 60, 180, 360 days). TC, total casein. (From Ref. 14.)

α_{s1}-casein could be attributed to the synergistic action of the residual rennet and the cell-bound proteinases of starter bacteria.

The RP-HPLC profile of the water-soluble fraction confirms the significant role of residual rennet in Feta cheese ripening (Fig. 2). Most of the identified peptides of that fraction originate from the N-terminal half of the α_{s1}-casein. Cleavage of Phe^{23}-Val^{24}, Phe^{32}-Arg^{33}, Leu^{98}-Leu^{99}, Leu^{101}-Lys^{102}, and Leu^{109}-Glu^{110} bonds could be attributed to chymosin action. β-Casein is the main substrate for plasmin activity, which is reflected in the production of γ-caseins from its C-terminal part. However, the two identified peptides originating from β-casein in Feta cheese extract apparently result from the cleavage of Leu^{190}-Tyr^{191} and Ile^{205}-Leu^{206} bonds by chymosin (30). Although the isolation of κ-casein peptides from cheese has not been reported, a peptide corresponding to κ-casein (f 96-105) was also detected in the water-soluble fraction of Feta cheese. It presumably originated from para-κ-casein and its formation could be the result of the action of lactococcal proteinase at Met^{95}-Ala^{96}, which exhibits the characteristics of a susceptible cleavage site for such an enzyme (40).

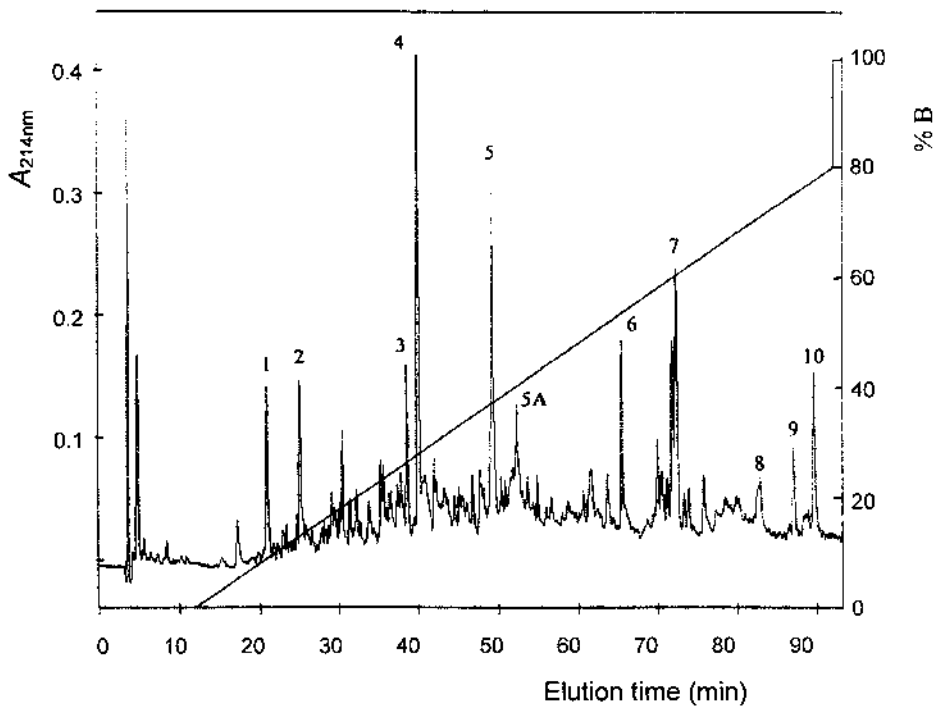

Figure 2 Reversed-phase HPLC profile of the water-soluble fraction of 6-month-old Feta showing the peaks collected and identified. Eluent A was 1 mL of trifluoroacetic acid (TFA)/L of deionized water. Eluent B was 0.9 mL of TFA, 399.1 mL of deionized water, and 600 mL of acetonitrile/L. Gradient: 0 to 10 min, eluent A; 10 to 90 min, 0 to 80% eluent B; 90 to 100 min, 100% eluent B. The flow rate was 0.8 mL/min. The absorbance of the eluate was monitored at 214 nm. Peak numbers correspond to the following compounds: 1, Tyr; 2, Phe; 3, α_{s1}-CN (f 4-14) and α_{s1}-CN (f 40-49); 4, α_{s1}-CN (f 1-14); 5, β-CN (f 164-180), α_{s1}-CN (f 102-109) and α_{s1}-CN (f 24-30); 5A, κ-CN (f 96-105) and α_{s1}-CN (f 91-98); 6, α_{s1}-CN (f 24-32); 7, β-CN (f 191-205); 8, α-LA; 9, β-LG (f 16-?); 10, β-LG. (From Ref. 30, courtesy of the Journal of Dairy Science.)

3. Small Peptides and Free Amino Acids

Important amounts of low-molecular-weight protein fragments (small peptides and free amino acids, FAA) are produced within 15–20 days. At the end of that period, more than 65% of the water-soluble nitrogen is soluble in 12% trichloroacetic acid (TCA) (12,14,31), indicating a significant accumulation of FAA and small peptides with 2–20 amino acid residues (41). FAA content increases by about 5 times during that period (20). It should be noted here that the FAA content of 1-day-old Feta is significantly different from zero (20). This is attributed to an accelerated amino acid release at the day of manufacture, when starter cultures are incubated for more than 30 min at temperatures favorable for their development and activity (42).

Because FAA release is attributed to the action of microbial peptidases (43), the starter culture seems responsible for the massive production of FAA during the first 15 days of ripening. However, the role of the NSLAB could not be overlooked. Sometimes, an insufficient thermal treatment of the cheese-milk, combined with the technology of Feta cheese (dry salting and further manipulations for 3–4 days), permits the curd surface to be contaminated with microorganisms of the cheese plant environment, and these can be the sources of NSLAB. The high temperature of the ripening room favors the development and proteolytic activity of both starter bacteria and NSLAB. Lactococcal peptidases are intracellular and their action indicates cell lysis (44). The high rate of FAA production during the first 2 weeks of ripening may be attributed to an early cell lysis, probably due to the low pH and high salt content of the curd.

After 20 days, the rate of proteolysis slows. Table 3 shows that the increase of the water-soluble and 12% TCA-soluble nitrogenous fraction (expressed as percentage of the total N content of the cheese) is quite slow (14,16,20,31). This is probably due to the low-temperature storage of Feta cheese and to the already mentioned diffusion of these compounds into the brine. On the other hand, the composition of the 12% TCA-soluble fraction continuously changes. This extract is enriched with peptides of a molecular weight lower than 600 Da and FAA (45); the content in peptides and FAA significantly increases with ripening time. The FAA content of 2-month-old Feta cheese varies from 2000 to 7000 mg/kg cheese. Leucine is the major FAA, ranging from 14% to 24% of the total, but levels of valine, lysine, and phenylalanine are also important (16,20,31). Another significant increase in FAA content occurs after 3 months of ripening, probably due to massive starter flora and NSLAB lysis and the availability of substrate (small peptides) for the action of the peptidases released (20).

4. Biogenic Amines

Biogenic amines in cheese are mainly generated from the decarboxylation of free amino acids by adventitious microorganisms rather than by starter bacteria (46). The sheep's and goat's milk used for Feta cheesemaking sometimes receives a thermal treatment below pasteurization conditions. Also, the manipulation of the curd during dry salting facilitates a contamination by various bacterial species, eventually possessing decarboxylating properties. This could, therefore, cause the production of high amounts of biogenic amines. However, investigations have shown that the average total amine content of Feta (390 mg/kg) is much lower than that reported for cheeses suspected in outbreaks of food poisoning. It seems, therefore, that the characteristic features of Feta (low pH, high salt content, ripening and storage in brine, not extended proteolysis) create an environment unfavorable for amine accumulation (20). Tyramine is the main biogenic amine in mature samples (about 42% of the total).

B. Lipolysis

Lipolysis is important for the development of the characteristic flavor of Feta cheese. Free fatty acid (FFA) content in mature cheese ranges from 1000 to 6000 mg/kg (16,18,47). The extent of lipolysis varies with the kind of rennet (homemade rennets are rich in lipases), the kinds of starter bacteria and NSLAB, the temperature in draining and dry salting, and so forth. High temperatures during draining enhance the lipolytic activity (13). Short-chain volatile acids (C_2-C_8) constitute 30–50% of the total FFA in mature cheese; among them, acetic acid is dominant at 29–47% of the total FFA (13,16,18,47–49).

C. Other Flavor Compounds

Due to the lack of pressing, the curd of Feta cheese retains a high moisture level and provides to the starter microorganisms and NSLAB enough lactose for fermentation. Quick lactose metabolism to lactate and a subsequent drop of the pH to about 4.6 is vital for the quality of the cheese (50). Later, the high acidity and salt content of the cheese create an environment unfavorable for the growth and metabolism of starter bacteria. However, NSLAB survive and may continue to metabolize lactose, citrate, and other cheese constituents to flavor compounds (2).

The main volatile compound of Feta is ethanol, followed by acetic acid, acetaldehyde, acetoin, and other short-chain alcohols, aldehydes, and ketones (22,49,51). High concentrations of ethanol (over 1000 mg/kg) in mature cheese were determined by dynamic headspace analysis. Ranges for acetaldehyde are 4–15 mg/kg; higher values are found in cheeses for which yogurt microorganisms were used as starter culture (18,22,25). Diacetyl is not found in mature Feta (18,22).

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Cheddar Cheese

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I. INTRODUCTION

Cheddar is a low-moisture hard-pressed cheese that originated in Somerset, England. The historical development of Cheddar from traditional farm production in the 16th century to industrial-scale manufacture has been reviewed by Rance (1). Cheddar cheese is produced extensively throughout the world today. The United States of America, the United Kingdom, Canada, Australia, Ireland, and New Zealand are the main producers (2,3). During 1999 the total production of Cheddar worldwide exceeded 1.9 million tons.

Cheddar cheese is produced by the coagulation of cow's milk with rennet. Acid production during manufacture is achieved by use of mesophilic type O cultures of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. The principal function of the starter is the uniform production of lactic acid at a predictable rate to ensure that quality cheese is produced. Control of lactic acid production during Cheddar manufacture is essential in ensuring that the level of chymosin retention in curd is optimum for flavor and texture development during maturation; curd strength is optimum for cutting and yield potential of milk is realized; moisture expulsion by syneresis proceeds at the correct rate and satisfactory moisture levels are achieved in the final cheese; and the dissolution of colloidal calcium phosphate is optimum for rheology and texture of the cheese (4). The starter culture in Cheddar manufacture also has an important role in the development of the typical flavor and aroma during maturation, and this is due mainly to proteolytic activity (5). Cheddar is matured for periods from 3 to 18 months, but extended maturation periods of up to 2 years or more may be used in production of specialist cheeses.

II. TECHNOLOGY OF MANUFACTURE

A. Quality of Milk for Cheddar Manufacture

The quality of milk used in Cheddar manufacture influences the cheese yield, the flavor, and the texture character of the final product. The most important milk constituents in terms of cheese yield and texture are the fat and casein content of the milk; the microbiological quality of the milk (6,7) and the presence of indigenous milk enzymes (8) have an impact on the ultimate flavor of the cheese.

The breed of cow, stage of lactation, and the type and level of feeding all influence milk composition (9). Seasonal changes in milk composition result mainly from lactational and dietary effects. The principal determinants of yield and quality of cheese are the fat and casein content of the milk. The sum of these two components is the principal determinant of cheese yield, and the ratio influences both the quality and composition of the final Cheddar. Milk for Cheddar manufacture in large commercial creameries is delivered by tanker from individual farms and is bulked and stored in silos prior to standardization and pasteurization. Extended storage of raw milk prior to manufacture can result in growth of psychrotropic organisms. These produce heat-stable lipolytic enzymes that survive pasteurization and can have a negative influence on flavor development during ripening (6,7). Seasonal variation in milk composition gives rise to significant changes in the balance of fat and protein and as a consequence of this, milk for Cheddar manufacture is generally standardized so that a casein to fat ratio of 0.70 is achieved. Failure to standardize can result in reductions in yield efficiency, lack of moisture control during manufacture, and production of substandard cheese that does not meet required specifications for fat in dry matter.

Following standardization, milk for Cheddar manufacture is pasteurized. Pasteurization effectively destroys pathogenic nonsporing bacteria, but spore-forming bacteria such as *Clostridium tyrobutyricum* can survive and potentially give rise to defective flavors and gas production in Cheddar in the later stages of maturation.

B. Basic Stages in Cheddar Manufacture

The basic steps in Cheddar manufacture are outlined in Fig. 1. The temperature profiles shown are typical, but processing times are related to scale of manufacture and differ among various commercial systems and traditional production. Manufacture of Cheddar

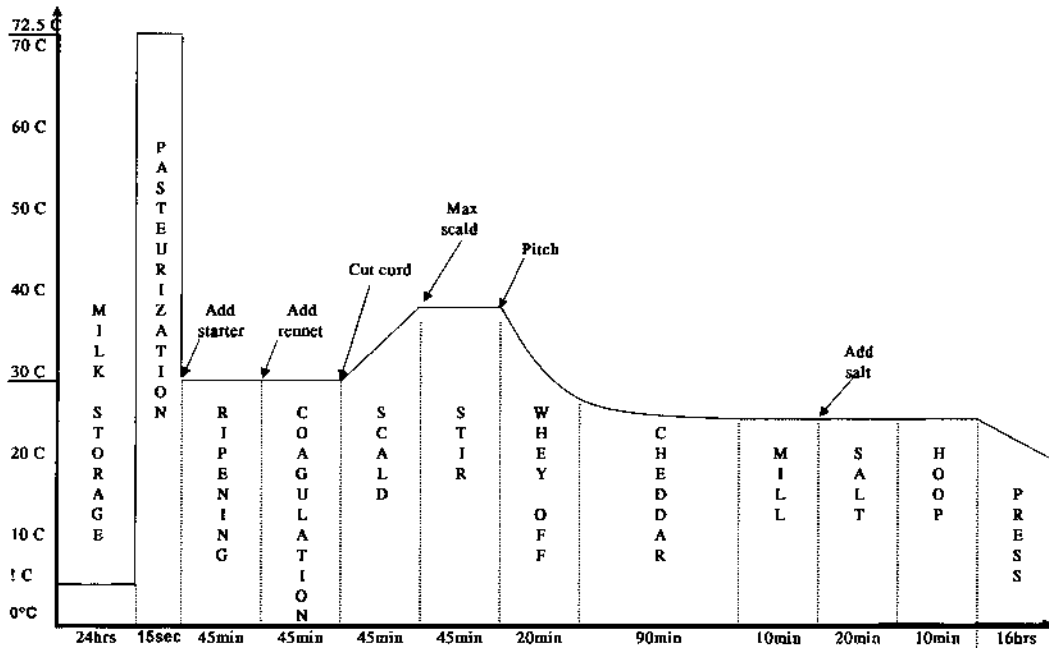

Figure 1 Typical process for traditional Cheddar manufacturer.

cheese is initiated by coagulation of starter acidified milk with chymosin or a suitable chymosin substitute (10,11). The chymosin destabilizes the colloidal suspension of casein micelles by hydrolysis of κ-casein (11). The release of the hydrophilic glycomacropeptide reduces steric hindrance and the surface micelle charge that results in micellar aggregation to form a gel (11). The resulting coagulum, which in Cheddar manufacture is formed at a temperature between 30 and 32°C and a pH of 6.4, comprises a continuous casein network in which fat is entrapped. The coagulum is then cut into small cubes and heat is applied with stirring to facilitate expulsion of moisture from the curd. As the temperature during scald is increased from 30 to 39°C over a period of 45 min, the curd particles begin to contract and expel moisture by a process termed syneresis.

Moisture expulsion is further enhanced by continued acid production by the starter and maintenance of the temperature at 39°C while stirring continues for a further 45 min. At an appropriate level of acidification and moisture reduction, the whey is removed from the curds by drainage. The pH of the whey at pitching is approximately 6.18 in traditional Cheddar manufacture. Approximately 90% of the starter culture added during Cheddar manufacture is retained in the curd following whey drainage.

Following drainage, the process of cheddaring begins. During the cheddaring period, the curds are encouraged to fuse by application of pressure. Acid development by the starter proceeds rapidly due to the high concentration of starter in the curd. Under the influence of heat, acid, and pressure, the curd particles mat together to form a solid mass. A central channel is cut in the fused curd to allow whey drainage in traditional manufacture. The curd mass is then cut into slabs which are turned and stacked at regular intervals in a controlled and incremental manner over a period of about 90 min. In modern mechanized systems, the process of cheddaring is highly automated and is achieved on a continuous belt system enclosed in tunnels (12). The perforated belt system allows whey drainage as the curd particles mat together and incorporates a mechanism for turning the curd.

Once the appropriate level of moisture, acidification, and structure is obtained the cheddared curd is milled and dry-salted. Addition of salt facilitates further reduction of moisture level in curd and inhibits acid production by the starter. In traditional manufacture the salted curd is transferred to molds for overnight pressing. However, in highly automated manufacturing systems the milled curd particles are vacuumed into tower block formers (12). The tower acts as a pressing system by virtue of the weight of curd in the vertical column. Whey is continually siphoned off as the curd mass proceeds down the tower. A guillotine system at the base of the tower called a block former cuts a 20 kg block from the stack at regular intervals and the block is transferred to a vacuum packing system. The pressed cheese is matured at temperatures ranging from 5 to 12°C. An important factor in the control of Cheddar quality is the rate and extent of acid production in the vat because this largely determines the final pH and basic structure of the cheese (13). The pH at which the whey is drained from the curd is a critical control point in determining Cheddar quality because this influences the calcium content of the final cheese and the proportion of chymosin retained in curd. Stringent monitoring and control of starter activity during manufacture is essential.

Cheddar cheese is matured for periods of from 3 to 18 months, but extended maturation periods of up to 2 years or more may be used occasionally in production of specialist cheeses. The length of the maturation period determines the flavor and texture character of the cheese. Cheddar produced to a mild recipe would be marketed at 3 months when the flavor is mild and creamy and the young cheese has a pliable texture. A strong flavor intensity, and a texture that is firm and crumbly would be characteristic of a cheese produced to a mature recipe, which would be ripened in excess of 12 months.

Compositional standards for Cheddar stipulate that the cheese should contain 39% or less moisture and more than 48% fat in dry matter. Cheddar produced for the mature or extra-mature market would be expected to have a moisture content of 33–35%, a fat in dry matter content of 52–54%, a salt content of 1.6–1.8%, and a pH of 4.95–5.25. A higher-moisture cheese, 37–38%, would be ripened for 3–4 months and marketed as mild Cheddar. Grading systems based on compositional parameters have been used for many years to predict quality (13), but more recently attribute grading by sensory profiling flavor character has been used to supplement compositional grading. The attribute grading system has two key elements: a descriptive analysis to determine the sensory attributes of the cheeses and a fitness-for-purpose judgment to determine whether the attribute profile meets the final Cheddar specification (14).

C. Cheddar Maturation

Flavor and texture in Cheddar develop over an extended maturation period. Proteolysis is the most extensive biochemical event occurring during ripening and lipolysis is limited in contrast (10). The proteolytic process is initiated by the hydrolysis of α_{s1}-casein by chymosin originating in the coagulant and the degradation of β-casein by the micelle-associated plasmin (10). Approximately 6% of the chymosin added to milk for Cheddar manufacture is retained in the curd. Chymosin retention in Cheddar is determined by the pH of whey at drainage and activity during ripening is controlled by the pH of the cheese (10). Residual chymosin in Cheddar rapidly hydrolyzes α_{s1}-casein at the Phe_{23}-Phe_{24} bond producing α_{s1}-casein (f1-23) and α_{s1}-casein (f24-199) (15). Further degradation of the α_{s1}-casein (f24-199) peptide by chymosin has been shown to generate the peptides α_{s1}-casein (f33-199), α_{s1}-casein (f102-199), and α_{s1}-casein (f110-199). Further hydrolysis of the α_{s1}-casein peptides is achieved by action of plasmin and the lactococcal cell envelope associated proteinase.

The indigenous milk proteinase, plasmin, is stable to pasteurization and is almost completely retained in curd during Cheddar manufacture to give a concentration of approximately 3–4.5 $\mu g\ g^{-1}$ curd (10). The main role of plasmin in Cheddar ripening is in the degradation of β-casein. β-Casein is more resistant to hydrolysis than α-$_{s1}$ casein, and only 50% of β-casein is degraded in mature Cheddar (15). The primary cleavage sites of plasmin on β-casein are Lys_{28}-Lys_{29}, Lys_{105}-Gln_{106}, and Lys_{107}-Glu_{108}. Hydrolysis at these sites yields the peptides β-casein (f29-209), β-casein (f106-209), and β-casein (f108-209) (13). Plasmin is also responsible for the cleavage of the α_{s1}-casein (f24-199) at the Lys_{103}-Tyr_{104} and $Lys_{105} - Val_{106}$, yielding α_{s1}-casein (f104-199) and α_{s1}-casein (f106-199) (15).

The initial degradation of the caseins in the first weeks of ripening results in the softening of the rubbery texture of young Cheddar curd, but the large peptides generated are tasteless. Further degradation of the casein-derived peptides by the starter and non-starter lactic acid bacteria (NSLAB) results in the formation of small peptides, amino acids, and volatile compounds derived from amino acid catabolism that contribute to flavor.

D. Components of Cheddar Flavor

Development of Cheddar flavor cannot be associated with an individual potent chemical but results from the generation of a number of components during ripening. The production of these flavor compounds must be balanced, and excessive production of one component can lead to development of off-flavors (16).

Peptides and amino acids contribute to the background savory flavor of Cheddar, but specific peptides can impart undesirable bitter flavours. Volatile aroma compounds have potential to add specific aroma and flavor notes to Cheddar. These are derived mainly from catabolism of amino acids, but also originate from lipolysis. Origins of flavor components in Cheddar have been studied extensively and several comprehensive reviews have been published (16–18).

Volatile components contributing to flavor include fatty acids, ketones, lactones, esters, and volatile sulfur compounds. 3-Methylbutanoic acid, derived from catabolism of leucine, has a rancid, cheesy, sweaty odor that may contribute to mature Cheddar aroma. Enhancement of this component in young Cheddar using α-ketoacids has been shown to be associated with an increase in the intensity of Cheddar aroma (19). Butyric acid, derived from lipolysis, has a cheesy, sweaty odor and is considered to be an important component of Cheddar flavor. Methional present in Cheddar has a boiled potato–like aroma; methanethiol, DMDS, and DMTS add garlic notes to the flavor of mature Cheddar. The aldehyde 3-methylbutanal at high concentrations imparts unclean harsh flavor in Cheddar but at low levels gives a pleasant fruity flavor. Ethylbutyrate, if present at excessively high levels, may cause a fruity flavor defect in Cheddar. Indole and skatole, products of the catabolism of phenylalanine and other aromatic amino acid metabolites, are responsible for unclean and barnyard flavors in Cheddar.

E. Bitterness

Bitterness in Cheddar is associated with the accumulation of hydrophobic peptides, which are formed by action of the coagulant and starter proteinases (15). Bitter peptides can be derived from both α_s- and β-caseins. Chymosin is important in the production of bitter peptides, because residual coagulant in Cheddar releases extremely hydrophobic peptides from β-casein. Cutting the coagulum at low pH in Cheddar manufacture results in excessive retention of chymosin and the development of bitterness (15). A low salt content in the final cheese will enhance chymosin activity and result in the accumulation of bitter peptides. The overall level of bitterness in Cheddar will depend on the relative rates at which bitter peptides are formed and degraded to nonbitter peptides (15). Certain *Lactococcus lactis* strains are associated with development of bitterness in Cheddar cheese. The total proteolytic activity of the starter strains can have a significant effect on development of bitterness. Strains for Cheddar production in New Zealand are carefully selected for specificity of lactococcal CEP, and mixed strain starters normally include one proteinase-negative strain in order to limit total proteolytic activity and control bitterness (20).

III. LACTIC ACID BACTERIA IN MANUFACTURE AND RIPENING OF CHEDDAR CHEESE

Cheddar cheese contains approximately 10^8 viable lactic acid bacteria (LAB)/g curd throughout the ripening period. The LAB present may have been added deliberated by the cheesemaker either as the primary starter culture or as a secondary adjunct culture during manufacture or alternatively may have entered the product adventitiously from the milk and other sources during the manufacturing process. Unlike the starter and adjunct cultures that are carefully selected, the cheesemaker currently is unable to control the diversity and development of the adventitious nonstarter population. The identity and

characteristics of these distinctive lactic acid bacterial populations will be considered in the following sections.

A. Starter Cultures

Starter cultures containing lactic acid bacteria have a definitive role in the manufacture of cheese and fermented dairy products (21). Starter cultures used in dairy fermentations are selected for rapid lactic acid formation and their ability to contribute to the development of the aroma, flavor and texture characteristics of the product, through a diverse range of metabolic attributes. For large-scale Cheddar manufacture in commercial plants, consistency of acid production throughout the cheesemaking process is essential, and the strains used are selected rigorously for bacteriophage resistance, salt sensitivity, acidification capabilities, and contribution to flavor formation. Two types of starter systems, bulk starter and direct vat inoculation, are used widely in Cheddar manufacture with the majority of production utilizing a multiple-strain starter comprising a mixture of at least two or three strains. The LAB used in type O starter cultures for Cheddar cheese manufacture are homofermentative mesophilic strains of *Lactococcus lactis* that are unable to ferment citrate and exhibit optimal growth at temperatures in the range 26–30°C (21–23). These O-type starter cultures are mixtures of strains of only *Lactococcus lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris*. Mesophilic mixed-strain starters for other fermented dairy products comprise different combinations of citrate-utilizing and citrate-negative strains of biovars of the two *Lc. lactis* subspecies and *Leuconostoc* spp.

B. Secondary Cultures

Many cheeses contain, in addition to the starter population, a secondary microflora that may have been added deliberately as defined adjunct cultures or may have developed as a consequence of adventitious contamination.

1. Adjunct Cultures

The production of Cheddar-type cheese, unlike many other cheese varieties, does not require the use of secondary cultures. However, adjunct cultures offer the means of improving and accelerating flavor development in a controlled manner (24,25). Adjuncts evaluated in Cheddar cheese production include both viable and attenuated preparations of *Lactococcus* spp. and *Lactobacillus* spp. (26).

Attenuated preparations, produced by physical, chemical, or enzymic treatments, are unable to grow in the curd but enhance enzymic activities during ripening (27). Evaluation of Cheddar cheese made with attenuated adjunct strains of *Lb. helveticus* and *Lb. casei* indicated improved sensory and textural characteristics (28–31). Thermophilic *Lactobacillus* spp. (*Lb. helveticus*, *Lb. delbruckii*) similarly do not proliferate significantly during maturation but their inclusion can improve flavor acceptability (32,33). However, mesophilic *Lactobacillus* adjuncts do grow in Cheddar curd during ripening and several studies on their use have been published (26). In the earliest studies (34,35) a reference flora that was composed of groups of nonstarter bacteria isolated from cheese and raw milk was added and shown to accelerate and intensify flavor development. Subsequent studies (36–46) specifically investigated the effects of individual strains of *Lactobacillus* spp. as adjunct cultures and recently the effects of combinations of adjuncts have been assessed (47). Adjunct cultures studied have, in general, been restricted to strains of *Lb. casei*, *Lb. paracasei* subsp.

paracasei, *Lb. curvatus*, *Lb. rhamnosus*, and *Lb. brevis*. These species also tend to dominate the cheese nonstarter flora, but the inclusion of adjunct strains is a specific strategy in flavor control. The selection thus cannot be random but must be based on the metabolic attributes exhibited by the strains. Typically, the inclusion of adjunct strains of lactobacilli has been shown to result in improved flavor intensity, increased aroma, and accelerated ripening, and although primary proteolysis was not affected by the adjunct cultures, the levels of small peptides and free amino acids were higher than in the control cheese. The same volatiles were present in both the control and adjunct-containing cheeses but their relative concentrations differed significantly. The inclusion of inappropriate adjunct strains, can, however, result in the occurrence of textural defects and development of undesirable off-flavors (36,38,42). An alternative strategy to accelerate cheese ripening is the use of bacteriocin-producing lactococcal adjunct cultures (48). The bacteriocins released by this strain increased the rate of starter cell lysis during ripening and in consequence the adjunct-containing cheese had elevated free amino acid concentrations and higher sensory grading scores. The potential for the inclusion of adjunct cultures with probiotic properties has also been evaluated because Cheddar cheese appears to offer some advantages over yogurt-type products in the delivery of viable probiotics to the human gastrointestinal tract (49). The dairy industry is continually examining opportunities for expanding the diversity of its product range, and it is inevitable that the range of adjunct cultures employed in the production of fermented dairy products will continue to expand as organisms with beneficial characteristics are identified (50).

2. Nonstarter Lactic Acid Bacteria (NSLAB)

The industrial-scale manufacture of Cheddar cheese is a rigorously controlled process that utilizes pasteurized milk in dedicated plants under hygienic conditions. However, despite the application of stringent precautions during cheesemaking, a secondary nonstarter bacterial population develops in the curd from adventitious LAB contaminants present initially in the milk, dairy plant, and surrounding environment. The nonstarter lactic acid bacteria (NSLAB) are initially present at levels of 10^2–10^3 cells g^{-1} in factory-made Cheddar cheese immediately on removal from the press (26). The population increases to 10^6–10^8 NSLAB g^{-1} after maturation for 3 months and remains at this level throughout the remainder of the ripening period (26,51). This nonstarter population dominates the viable bacterial flora of Cheddar cheese during most of the maturation period. Although the overall size of the NSLAB population remains relatively stable, pronounced shifts in the species complement and strain profile occur during ripening (52,53). Because the NSLAB are not added intentionally, their presence and activities introduce a variability into the process that is outside the control of the cheesemaker. Differences in the NSLAB populations of cheeses produced in different production runs in the same factory have been reported (52–54), and this variation may contribute to between-batch variations in the quality of the cheese produced in a creamery. Although Cheddar cheese can develop full mature flavor in the absence of NSLAB, as demonstrated by cheesemaking in aseptic vats (55), the NSLAB are considered to add desirable flavor notes and reduce the harshness and bitterness associated with some starter cultures. However, despite positive contributions in ripening, some Cheddar defects are attributable to activities of the NSLAB. The racemization of L(+)-lactate to D,L-lactate by some strains can result in the formation of white spots of calcium lactate crystals in the mature cheese (56), and textural defects associated with slit formation have been attributed to gas formation by heterofermentative lactobacilli (57,58).

The dominant nonstarter lactic acid bacteria in Cheddar cheese are mesophilic homo- and heterofermentative *Lactobacillus* species, although the occurrence of *Pediococcus* and *Leuconostoc* spp. has also been reported (51,53,59). Early studies on the microflora of Cheddar cheese (60 61 62) established the predominance of lactobacilli, and although the nonstarter population is heterogeneous and varies widely, studies on NSLAB in Cheddar produced in several different countries around the world consistently report the dominance of *Lb. paracasei*, *Lb. plantarum*, *Lb. curvatus*, and *Lb. casei* (52,53,63–66). *Lactobacillus rhamnosus* is prevalent, alongside *Lb. paracasei*, in Cheddar produced in New Zealand (47). Several other species have also been detected in commercially manufactured Cheddar cheese (67), although the number of species present in a cheese decreases during maturation (52,53). Genotypic (65), phenotypic (53), and serological (61) analysis has indicated that the NSLAB population can comprise 1 to 13 different strains. The variability of the NSLAB population, both within and between factories, is a consequence of the diverse origins of the lactobacilli and manufacturing practices. High-quality raw milk contains approximately 10^2 lactobacilli ml^{-1}, some of which may not be killed by HTST pasteurization (68,69), although Martley and Crow (70) argued that the milk was unlikely to be the principal source of NSLAB. They proposed that the manufacturing equipment was the more probable contamination site, and recently it has been reported that NSLAB were isolated from several surfaces in the dairy environment, including floors, drains, cleaning vats, hoops, and vacuum packaging equipment (71). Organisms in biofilms were able to survive cleaning and sanitizing regimens, and it was demonstrated experimentally (71) that lactobacilli added to the cheese milk could form biofilms on the cheesemaking equipment and persisted in a subsequent batch of cheese made after the plant had been cleaned. Studies of the NSLAB populations of cheeses made in a single plant over an extended period of time, however, did not detect the repeated recurrence of specific strains (52,53), suggesting that if the contamination was from within the plant it was likely that the originating source was at a low level or sporadic. Serological typing methods, in addition to identifying the milk as a source of NSLAB in cheese, confirmed that airborne lactobacilli were also detectable in the cheese (72).

C. Factors Affecting the Growth and Survival of LAB in Cheese

The Cheddar cheese ecosystem is typically a low-pH, low-oxygen, high-salinity environment in which the LAB are heterogeneously dispersed as microcolonies (73). During Cheddar cheese manufacture, the numbers of starter lactococci increase to a maximum of 10^8–10^9 g^{-1} at salting, although the actual numbers will depend on the lactococcal strains used, as some may be adversely affected at the cooking temperature (~39°C), which is toward the upper limits for growth of mesophilic lactococci. *Lactococcus lactis* subsp. *cremoris* is the more heat-sensitive of the two subspecies (21). The number of viable starter cells declines after salting, at a rate that is strain-dependent (74). *Lc. lactis* subsp. *cremoris* is also more sensitive to salt and will not grow at 2% salinity, whereas *Lc. lactis* subsp. *lactis* will grow in the presence of 4% (w/v) sodium chloride. A major role of the starter is the conversion of lactose to lactic acid. Salting reduces the rate of lactose metabolism and acidification by the starter and the optimal salt in moisture ratio for good-quality Cheddar is between 4 and 6. The S/M ratio is a principal determinant of the rate at which the lactococci convert the lactose into lactate and ultimately impacts on the final pH of the cheese (75). The NSLAB are more salt tolerant than the starter lactococci and are not inhibited until the salinity is approximately 10% (w/v).

The commencement of the decline in viable lactococcal numbers post-salting is detectable at pressing (74), and this decrease in the population continues, at an increased

rate, in the initial stages of ripening. The rate of starter autolysis is strain-dependent and is important in controlling cheese ripening and flavor development through the release of intracellular enzymes into the cheese curd. Although usually beneficial, autolysis, if too rapid, can have detrimental effects (e.g., reduced acid formation). Optimal flavor development requires autolysis to occur in a controlled manner, and starter culture mixes can include autolytic strains that enhance ripening but have no major role in primary acid production. N-acetylmuramidase is the principal autolysin in *Lc. lactis*, although differences in the level of this enzyme do not account for the large variations in the extent of autolysis evident in commercial *Lc. lactis* strains. Other enzymes capable of hydrolyzing the cell wall are present in starter lactococci (76). However, sudden unanticipated declines in the starter population can usually be attributed to bacteriophage infection. Lactic acid bacteria can vary widely in their susceptibility to phage infection and four different mechanisms of resistance have been described—namely, abortive infection, adsorption inhibition, inhibition of DNA infection, and systems for DNA restriction and modification (77). The use of bacteriophage-resistant strains in starter cultures is an important control measure, although the prevention of phage infection necessitates the implementation of control measures throughout the manufacturing process if the associated inhibition of acid production and product quality impairment is to be avoided. Typical strategies adopted include the selection, preparation, and manufacture of virus-free or resistant cultures, culture rotation, and measures to prevent the entry and spread of phage in the processing facility.

In contrast to the starter lactococci, the nonstarter and adjunct lactobacilli are not adversely affected by ambient physiological conditions in the cheese curd and are thus able to proliferate during maturation. NSLAB have a generation time of approximately 8.5 days in cheese ripened at 6°C (64), and viable cells are recoverable from cheese stored at 10°C for 3 years. The development of the NSLAB population from the initial adventitious contaminants in the pressed curd or the proliferation of adjunct strains in the cheese and their subsequent effects on cheese character are ultimately dependent on the ability of the individual lactobacilli to grow under the physiological and nutritional conditions apertaining in the cheese matrix. The growth rate and final population density are not affected significantly over the pH range and the salt and moisture levels that normally occur in the curd during Cheddar cheese manufacture (78). The rate of growth of adventitious lactobacilli is temperature dependent, although ripening temperature had little influence on the overall number of lactobacilli in the cheese after a ripening period of 9 months. However, rapid block cooling and ripening at a reduced temperature of 8°C reduced their growth rate (79). In cheese ripened at 1°C, the NSLAB population was 3 log cycles lower than in a cheese ripened at 8°C (80). There is evidence that stacked 20 kg blocks of cheese may take an extended period to cool to ambient temperature, and in consequence some manufacturers have introduced a precooling of the blocks prior to palleting (26) because it has been shown (79,81) that rapid cooling to less than 10°C after pressing retards NSLAB growth and reduces the risk of off-flavor development.

The proliferation of nonstarter lactobacilli during ripening is also dependent on their ability to utilize the substrates that are initially present, or become available, in the curd. The energy sources potentially available include milk constituents, bacterial metabolites, and cell lysis products. It has been confirmed (82) that there are pronounced interspecies and strain differences in the range of carbon substrates metabolized by the NSLAB. Sugars are used widely among the lactobacilli, including those potentially derived from nucleic acid and casein deglycosylation. Generally, bacterial catabolites and lipids were less effective substrates (82), although glycoproteins and glycolipids occurring in the milk fat globule membrane support the growth of some nonstarter strains (26). Importantly, peptides and amino acids are catabolized by lactobacilli provided that a keto acid acceptor is present to

facilitate the aminotransferase-mediated degradative pathway (82–84). Differences in the metabolic capabilities of individual nonstarter strains are consistent with the observed dynamic state and strain succession in the NSLAB population during ripening (52,53,65). It is unrealistic to expect that the nonstarter population would remain constant during an extended ripening period. The species and strain profile of the population will inevitably fluctuate as those strains best able to utilize the available growth substrates become dominant and subsequently then decline when that carbon source is depleted.

The disappearance of nonstarter strains during ripening is consistent with autolysis taking place. The evidence to date is, however, inconclusive and as with the starter lactococci the extent of autolysis may be strain-dependent. Kiernan et al. (85) were unable to find evidence for the autolysis of mesophilic lactobacilli during ripening, but there are reports that the thermophilic species *Lb. helveticus* is susceptible to autolysis (85,86). Autolysates of starter lactococci and adventitious lactobacilli can increase the diversity of the nonstarter population through the provision of growth substrates (86,87) and accelerate ripening because of the release of proteolytic enzymes that contribute in the ripening process (88). Alternatively, the failure of a strain to persist throughout ripening is related to its susceptibility to antimicrobial compounds formed by other LAB in the curd. Antimicrobial substances produced by some of the starter lactococcal and nonstarter *Lactobacillus* strains include nonspecific acidic metabolites such as lactate, acetate, and formate and other nonacidic catabolites among which hydrogen peroxide, carbon dioxide, and diacetyl are inhibitory (89,90). Many lactobacilli and lactococci also are able to form bacteriocins that either may have a broad spectrum of activity or, alternatively, exhibit activity against closely related LAB (90,91). The inclusion of starter LAB that are able to form these antagonistic proteins and peptides offers a means of suppressing the growth of undesirable bacteria during ripening, and cheesemaking trials have been conducted using bacteriocin-forming strains to control the growth of both foodborne pathogens and NSLAB (92–94).

D. Characteristics of LAB Associated with Cheddar Cheese

The lactic acid bacteria comprise a diverse group of gram-positive, nonsporing, aerotolerant rod or coccoid microorganisms that produce lactic acid as the major fermentation product of carbohydrates. As a consequence of numerous taxonomic revisions, 16 genera are currently regarded as being lactic acid bacteria (95). Of these, species of *Lactococcus* and *Lactobacillus* are predominant in Cheddar cheese made with an O-type starter culture. *Pediococcus* and *Leuconostoc* spp. are infrequently reported to occur in the nonstarter population, and the use of *Enterococcus* spp. as a flavor-producing adjunct has been advocated, although this does raise potential food safety issues (96). Enterococci may be present in cheese manufactured from unpasteurized milk. These three latter genera are not, however, regarded as being constituent members of a mesophilic mixed-strain type-O starter culture.

Although several species of lactococci (formerly group N streptococci) have been described, it is only the two subspecies of *Lc. lactis* that are used in type-O starter cultures. The cells are coccoid in shape and occur singly, in pairs, or in chains. They are homofermentative and capable of growth at 10°C but not 45°C. *Lc lactis* subsp. *cremoris* can be distinguished from *Lc. lactis* subsp. *lactis* biochemically because it is unable to ferment maltose and ribose, does not hydrolyze arginine, and is unable to grow in the presence of 4% (w/v) NaCl.

Lactobacilli are used as adjunct cultures with the starter lactococci or are present as nonstarter adventitious contaminants in cheese made with O-type starters. The genus *Lactobacillus* is a genetically and physiologically diverse group of rod- or coccobacillus-

shaped bacteria that can be divided into three groups based on fermentation end products (97). Classic phenotypic identification is not always reliable with cheese isolates, and a polyphasic approach using combinations of phenotypic, genotypic, and chemotaxonomic criteria is now advocated for unequivocal species assignment (98). The ability of lactobacilli to grow in milk or cheese is not a ubiquitous trait among members of the genus.

1. Biochemical Activities

The principal functions of the starter are rapid acidification and the generation of flavor compounds during the ripening period. Lactic acid formation and the early stages of ripening are effected principally by the starter lactococci, and although starter enzymes are important in maturation the activities of the adjunct and adventitious lactobacilli contribute to proteolysis and lipolysis (58).

2. Lactose Metabolism

The dominant feature of LAB metabolism is substrate-level phosphorylation-mediated ATP generation from carbohydrate fermentation. Rapid lactose fermentation in lactococci is associated with the involvement of a phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) in which phosphoenolpyruvate is the phosphoryl donor. Most dairy starter *Lc. lactis* strains possess a lactose PTS so that lactose enters the cell as lactose phosphate, which is hydrolyzed by phospho-β-galactosidase into glucose and galactose-6-phosphate. Glucose is phosphorylated by glucokinase and metabolized by the Embden-Meyerhof homofermentative glycolysis pathway via pyruvic acid to lactate (Fig. 2). Galactose-6-phosphate enters glycolysis via the tagatose-6-phosphate pathway. An alternative mechanism of lactose uptake in LAB involves lactose permease as the carrier; the lactose is subsequently hydrolyzed to glucose and galactose by β-galactosidase (Fig. 2). Many LAB possess both a lactose PTS and lactose permease system for lactose metabolism (95).

There are two major routes for the intermediary metabolism of glucose in LAB (Fig. 2). The homofermentative pathway generates 2 moles of lactate and ATP for each mole of glucose utilized. The glycolysis (Embden-Meyerhof) pathway is used by all LAB in Cheddar cheese, with the exception of *Leuconostoc* spp. and heterofermentative lactobacilli. The alternative heterofermentative pathway utilized by these LAB yields one ATP and one mole each of lactate, ethanol, and CO_2 for each mole of glucose utilized. In some LAB that transport lactose with a permease, galactose is phosphorylated and transformed to glucose-6-phosphate by the Leloir pathway (95).

Regeneration of the pyridine nucleotide (NAD^+) occurs in the concomitant enzyme-mediated reduction of pyruvate to lactate by L- or D-lactate dehydrogenase. The proportion of the individual isomers formed by individual LAB strains is dependent on the possession of the appropriate lactate dehydrogenases and their respective activities, although some species possess an L-lactic acid racemase that converts L-lactic acid to the D-form. Lactococci produce only L-lactate. However, metabolites other than lactic acid may be formed from pyruvic acid in response to changes in environmental conditions or alternative electron acceptor availability (95,99).

3. Citrate Metabolism

Citrate is present in only low levels (0.15–0.2%) in milk and O-type starters do not include the citrate utilizing *Lc. lactis* subsp. *lactis* biovar *diacetylactis*. Diacetyl, an end product of citrate catabolism, however, is an important flavor component in Cheddar cheese (17).

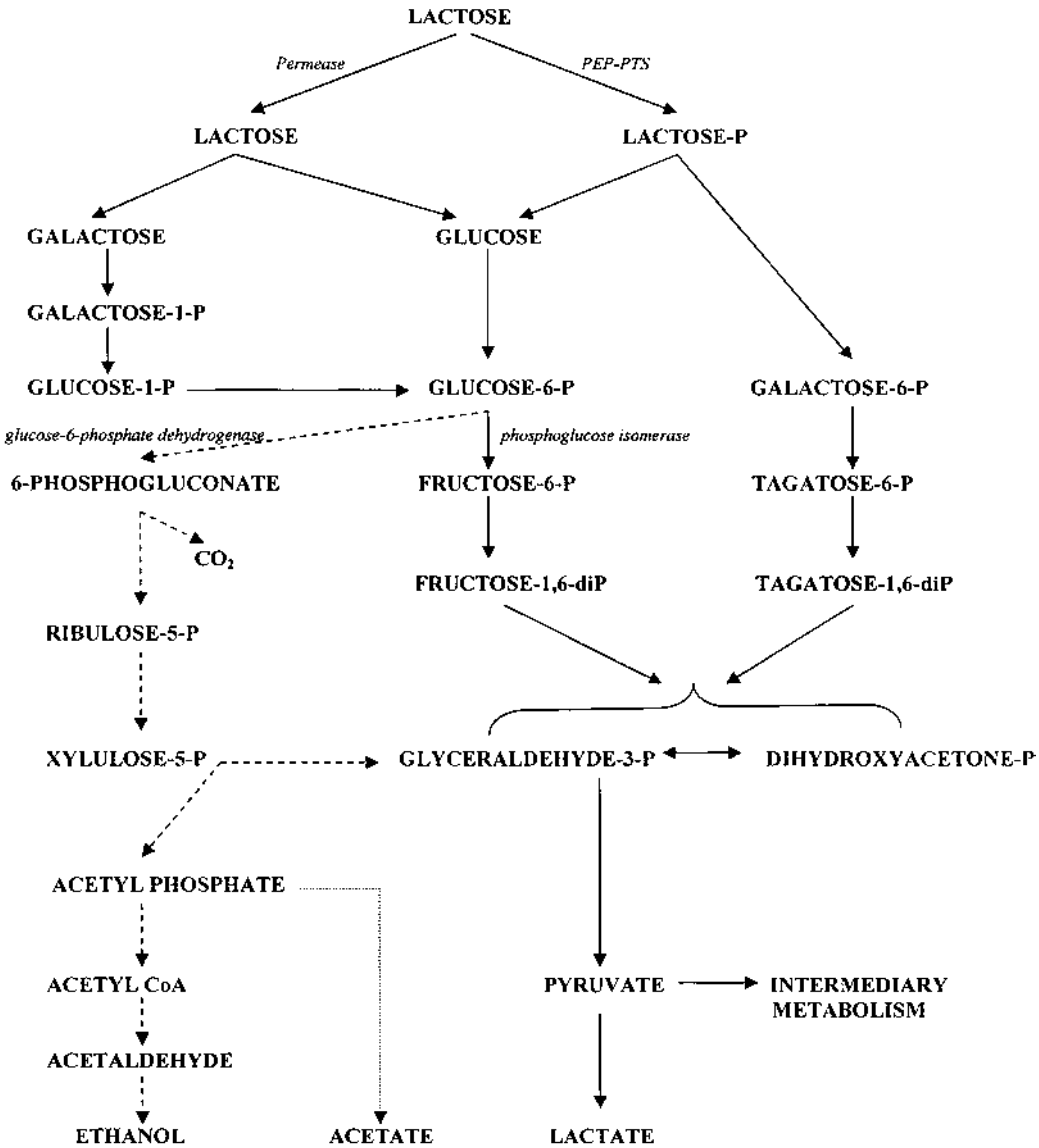

Figure 2 Pathway of lactose metabolism in cheese LAB. (Homofermentative and heterofermentative routes are indicated by intact and broken lines, respectively.)

Other end products of citrate metabolism—acetoin and 2,3-butanediol—are flavorless, although the carbon dioxide released may affect the texture of the cheese. In Cheddar cheese it is probable that the citrate is metabolized by the adventitious lactobacilli (100,101) although its value as an energy source for growth is equivocal (82,100).

4. Proteolysis

The levels of free amino acids in milk are insufficient to support the growth of auxotrophic starter LAB, although there are pronounced inter- and intraspecies differences in amino acid

requirements. The LAB possess a complex, albeit well-characterized, proteolytic system, the products of which enable the bacteria to meet their amino acid requirements from the hydrolysis of milk proteins. The peptides and amino acids released also contribute to, and act as precursors for, flavor development in cheese. In Cheddar cheese, primary proteolysis is affected by the added chymosin and endogenous milk enzymes; small peptides and free amino acids are released from the primary products by the action of the LAB proteolytic enzymes (102).

The proteolytic system of the lactococci has been examined extensively, many of the component enzymes have been purified and characterized, and the encoding genes sequenced (103–105). The proteolytic systems of other LAB are less well characterized, but several enzymes in many strains have been studied. In general these enzymes closely resemble and complement their lactococcal counterparts. The proteolytic complex of *Lc. lactis* comprises a cell-envelope–associated 180–190 kDa molecular mass extracellular serine proteinase, peptide and amino acid transport systems, and a diverse range of intracellular peptidolytic enzymes. The membrane-anchored proteinase (CEP, lactocepin, Prt) is the only extracellular lactococcal proteolytic enzyme, although the strain specificity toward caseins resulted in the initial classification of different proteinase groups (e.g., Prt I, Prt III). The specificity of the CEP from different *Lactococcus* strains on α_{s1}-, α_{s2}-, β-and κ-caseins has been established (102,103). The intracellular lactococcal proteinases are unlikely to be involved in peptide generation for growth in milk but may contribute to proteolysis in cheese as a consequence of starter cell lysis. Although less well studied, the principal cell wall–associated proteinase of lactobacilli resembles the lactococcal enzyme (102,103).

Casein degradation products are transported into the cell by specific amino acid transport systems, two distinct dipeptide/tripeptide systems (DtpT, DtpP), and an oligopeptide transport system (Opp) (103). The Opp transport system comprises two ATP-binding proteins, two membrane proteins, and a substrate-binding protein; it transports peptides containing four to eight amino acids. The Opp transport system, unlike the DtpT system, is essential for growth on casein: casein-derived oligopeptides, small enough to be transported by the Opp system, represent some 98% of the available nitrogen source for lactococci in milk.

The transported peptides are hydrolyzed intracellularly by a complex of peptidolytic enzymes with overlapping specificities. The types of peptide hydrolases present in LAB include both endopeptidases and N-terminal aminopeptidases, dipeptidases, and tripeptidases expressing exopeptidase activity. Although lactococci would not appear to possess a carboxypeptidase, this activity has been reported once in some lactobacilli (106). An extensive array of peptide hydrolases is utilized to release the amino acids essential for LAB growth. The general peptidases characterized to date in lactococci include two endopeptidases (PepF, PepO), a tripeptidase (PepT), a dipeptidase (PepV), and the metallo- and thiol-type aminopeptidases (PepN and PepC, respectively). In addition, lactococci possess peptidases with more specific functions, and these include glutamyl aminopeptidase (PepA), pyrrolidene carboxylpeptidase (PCP), and the four proline-specific enzymes—aminopeptidase P (PepP), proline iminopeptidase (prolylaminopeptidase, PepI), prolidase (PepQ), and a post-proline dipeptidyl peptidase (PepX). The peptidase profile of the lactobacilli studied is broadly similar, although PepA, PepP, and PepT have been reported only in *Lactococcus* spp. However, leucylaminopeptidase (PepL), a dipeptidase (PepD), prolinase (PepR), and the endopeptidases (PepE and PepG) conversely have been characterized only in some species of mesophilic and thermophilic lactobacilli. The properties of these and other unassigned LAB peptidases have been extensively reviewed (e.g., 102–105). Although approximately 20 different peptidase hydrolases have been characterized in

lactobacilli, the full complexity of the peptidolytic systems of most species of nonlactococcal LAB is not known. However, studies using diagnostic substrates indicate that most *Lactobacillus* spp., in common with *Lc. lactis*, possess a wide range of peptide hydrolase enzymes (67,107). The similarity of the peptidolytic enzyme systems in the two LAB genera is not unexpected as both groups of bacteria have evolved to survive and proliferate in an ecosystem in which the level of free amino acids is insufficient to sustain abundant growth.

The contribution of the lactococcal intracellular proteolytic enzymes in the ripening of Cheddar cheese may be greater than that of the enzymes of the nonstarter lactobacilli, in view of the greater tendency of starter cells to autolyse in the cheese during ripening. Neither bacterial group contributes significantly to primary proteolysis in Cheddar cheese, and although the contribution of the starter lactococci to secondary proteolysis is greater (43,108), the presence of both types of LAB is associated with increased proteolysis and higher levels of free amino acids in the cheese.

5. Amino Acid Catabolism

The starter lactococci and mesophilic lactobacilli, as discussed, are involved in the release of peptides and free amino acids from casein during the ripening of Cheddar cheese. Although these products of proteolysis contribute to the flavor, attempts to increase their formation by overexpression of enzyme activity or the addition of free amino acids to the curd at the manufacturing stage have not been successful in flavor enhancement. The implication, therefore, is that amino acid transformation rather than amino acid release is the key determinant in flavor formation. The catabolism of amino acids can result in the formation of many compounds that contribute to cheese flavor (16,109,110). Degradative mechanisms potentially include deamination, decarboxylation, desulfuration, oxidation, and reduction reactions resulting in the formation of amines, aldehydes, alcohols, indoles, carboxylic acids, and phenolic and sulfur-containing moieties (18,110).

Amino acid catabolism in starter lactococci occurs by two different pathways, depending on the nature of the amino acid. The degradation of branched-chain and aromatic amino acids is initiated by an α-ketoglutarate-dependent transamination mechanism. This appears to be the major pathway of amino acid breakdown in lactococci and the branched-chain and aromatic aminotransferase enzymes involved have been isolated and characterized both biochemically and genetically (18,105). The resultant α-ketoacids derived from the amino acids are subject to further enzymatic or chemical reactions. The end products of these reduction or decarboxylation reactions are hydroxyacids, aldehydes, and carboxylic acids (18). Nonenzymic degradations of ketoacids in cheese have also been reported (18). The degradation of sulfur-containing amino acid is also initiated by an elimination reaction catalyzed by the enzymes cystathionine-β-lyase and cystathionine-γ-lyase (111). Methional and methanethiol formed by the breakdown of methionine may be oxidized subsequently to dimethyldisulfide and dimethyltrisulfide.

Although the lactobacilli are less well studied than the lactococci, there is sufficient evidence to conclude that mechanisms of amino acid degradation in both are generally similar. The range of amino acid–converting enzymes in cheese lactobacilli is restricted (18,111). Amino acids are metabolized in the presence of α-ketoglutarate and a range of volatile catabolites are formed (83,84). Branched-chain (84,112), aromatic (84,113–115), and methionine (84,115–117), aminotransferase, and cystathionine lyase activities (84,117) have been detected in nonstarter lactobacilli, and although some aminotransferase characteristics have been determined (116–118), only cystathionine-γ-lyase from a cheese strain of *Lb. fermentum* has been purified and characterized (119). Amino acid decarboxylating lactobacilli have also been isolated from cheese (120).

Aminotransferases are intracellular enzymes, but cell lysates retain only limited activity under conditions prevailing in ripening Cheddar cheese (118). However, in growth studies, enzymes involved in amino release and turnover retained activity under cheese conditions provided that cell integrity was maintained (118). The aminotransferase-mediated conversion of methionine to thiols also proceeds more slowly under cheese conditions with extracts than with intact LAB cells (115). As the apparent stability and effectiveness of amino acid catabolizing enzymes released from lysed cells is less than in intact viable cells, the impact of lactobacilli on flavor formation during ripening may be diminished as a consequence of extensive cell lysis.

6. Lipolysis

The limited lipolysis that occurs during the ripening of Cheddar cheese manufactured from pasteurized milk is probably effected by the lipolytic enzymes of the starter lactococci and nonstarter lactobacilli, although LAB are generally only weakly lipolytic. Screening studies using natural substrates, triglycerides, and synthetic chromogenic substrates (58) have confirmed the presence of lipase (121–123) and esterase activities in mesophilic (67,123–125) and thermophilic (126,127) dairy lactobacilli. In general the enzymes were located intracellularly, and activities were strain specific but tended to be higher in thermophilic than mesophilic strains. In the majority of strains examined, activities increased as the carbon chain length of the fatty acid decreased. The only lipase purified is a 65 kDa molecular mass intracellular enzyme produced by *Lb. plantarum* (128). The enzyme hydrolyzed the triglycerides trilaurin and tripalmitin but was most active against tributyrin. The purified enzyme expressed optimal activity at pH 7.5 and 35°C but retained 45% of its maximum activity at pH 5 and 15°C.

LAB esterases have also been purified and characterized. The serine tributyrin esterase of both *Lc. lactis* subspecies has a subunit molecular mass of 29 kDa and optimal activity at pH 7–8 (129,130). The enzyme was active on chromogenic ester substrates having a fatty acid chain length of 2–12 carbon atoms. The gene encoding the major intracellular tributyrin esterase of *Lc. lactis* has been cloned and characterized (131). Intracellular esterases formed by *Lb. plantarum* (132,133), *Lb. casei* (134), *Lb. fermentum* (135), and *Lb. helveticus* (136) have also been purified and characterized. The estimated molecular masses ranged from 70 to 105 kDa (subunit mass 25–40 kDa), but many of the enzyme properties were similar. They are all serine-dependent enzymes that are inhibited by PMSF. Activities were maximal with butyrate ester substrates at a temperature of 34–40°C and at a pH of 7–8. Reduced activity was retained at pH5, at lower ripening temperatures, and in the presence of NaCl. The response of esterase activity to the effects of salt, temperature, and pH is strain-dependent (137), but retention of this activity during ripening may have important consequences for flavor as a result of both lipolysis and ester formation.

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15

Semihard Scandinavian Cheeses Made with Mesophilic DL-Starter

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I. INTRODUCTION

Several semihard cheese varieties are produced from pasteurized bovine milk in the Scandinavian countries, Denmark, Norway, and Sweden (Table 1). The Danish cheeses are often ripened with a smear surface microflora, giving them a special flavor note from sulfuric aroma compounds. These cheeses are among the semihard cheeses with the highest moisture contents. Propionic acid bacteria are used in combination with mesophilic starter in some Norwegian and Swedish varieties that have a smooth, sliceable texture with large round eyes and sweet, nutty, and sharp flavor components. Several cheese varieties, especially from Sweden, are ripened by no other bacteria than the mesophilic starter and lactobacilli from the environment. These cheeses have a mild to aromatic flavor, often with a sweet note and a pronounced flavor of diacetyl, if they are consumed, as most of them are, at a quite young age (from 2 to 6 months). Older cheeses develop a more aromatic and mature flavor. Those cheeses all have a semihard, sliceable texture that easily melts in the mouth. Much Scandinavian cheese is consumed sliced on bread.

Scandinavia has a long history of cheesemaking that can be traced to at least the 12th or 13th century. Milk production and cheesemaking may have been crucial for human survival in the northern parts of Europe. In many areas situated too far north for successful grain and vegetable production, it was still possible to have milk animals. The cows did not give milk during winter, and cheese manufacturing was a way to preserve the nutrients for wintertime. Production of open-texture cheese from renneted curd was described in literature by monks in mid-Sweden during the 16th century. Semihard cheeses that have their origin in this production are today Esrom and Havarti in Denmark as well as Svecia, Präst, and Hushållsot in Sweden.

During a period about 200 to 300 years ago, the Swiss cheese with a closed texture and large round eyes became very popular in Europe. Swiss cheesemakers were invited to teach local cheesemakers all over Europe how to make cheese with the Swiss method. The cheeses never turned out exactly the same at a new place, and several new cheese varieties were created. The Swiss cheesemakers that came to Scandinavia met some unexpected problems, and the cheeses sometimes needed 5 to 7 years of work to be developed into attractive cheese varieties. During the first trials, no or very poor acidification occurred

Table 1 Scandinavian Semihard Cheese Varieties Made from Bovine Milk Using Calf Rennet and Mesophilic DL-Starter

Name	MNFS %	FDM %	Adjunct culture	Country
Open texture				
Esruom	62	45	*Brevibacterium linens*	DK
Havarti	58–60	60, 45, 30	*Brevibacterium linens*[a]	DK
Hushållsost	57–60	45, 30	—	SE
Svecia	57–59	55, 45, 30	—	SE
Prästost	55–58	50, 30	—	SE
Round-eyed				
Danbo	60–62	45, 30, 20	*Brevibacterium linens*[a]	DK
Samsø	58–60	45, 30	—	DK
Herrgård	53–57	45, 40, 30	—	SE
Kadett/Västan	56–59	20, 10	Heat treated *Lb. helveticus*	SE
Norviega	58–59	45	—	NO
Jarlsberg	54–55	45	*P. freudreichii shermanii*	NO
Grevé	54–57	45	*P. freudreichii shermanii*	SE
Magré	55–57	30	*P. freudreichii shermanii*	SE

FDM %, fat in dry matter as % (w/w) in cheese
MNFS %, Moisture in non-fat substance as % (w/w) in cheese
Lb., *Lactobacillus*, *P.*, *Propionibacterium*
[a] Varieties without smear bacteria are also made.

and the cheeses did not ripen. The explanation could be found in the natural microbial flora that did not contain many thermophilic bacteria with the ability to survive the high cooking temperatures used in the Swiss recipes, and starter cultures were not used at this time. The environment around milk and cheese production was much colder than in southern Europe and almost never high enough to stimulate growth of thermophilic bacteria. A mesophilic microbial flora dominated completely. New cheese varieties were developed by decreasing the production temperatures, but to obtain larger eyes, somewhat higher temperatures than earlier were used. Two cheese varieties—Herrgård in Sweden and Samsø in Denmark—are the results of this early meeting of Swiss and Scandinavian culture.

The cheese Herrgård does not contain any propionic acid bacteria (PAB), but descriptions of it in early literature reveal that they were present at low numbers in those days. PAB were never added but originated from the raw milk microflora, and they probably disappeared with the introduction of pasteurization of the cheese milk. Experiments were made to add them as a culture, leading first to the Norwegian cheese Jarlsberg and later to the Swedish cheese Grevé as well as to the Danish cheese Svenbo—the latter, though, with thermophilic culture in addition to the mesophilic starter. Typical for those Scandinavian cheese varieties is the specific combination of PAB and mesophilic undefined starter and *Lactobacillus paracasei* as the main nonstarter lactic acid bacteria (NSLAB).

Danish cheeses with small round eyes were developed using production methods that were introduced by Dutch farmers and cheesemakers living in Denmark. Today, these kinds of cheeses commonly are made using smear surface ripening in Denmark, which is in contradiction to corresponding Dutch cheese varieties, and the moisture content is typically

higher in the Danish cheeses. The rather soft semihard cheese Danbo is the most common variety.

Reduced-fat cheese has been made for a long time in Scandinavia. The early varieties were made as low-budget cheese and were developed during the time when butterfat was considered to be the most valuable part of the milk. These low-fat cheese varieties have a fat in dry matter (FDM) content of 20% or 30%, and the properties of the latter may be quite similar to some of the normal fat cheese varieties with 45% FDM. However, their quality was not always as high as it was for the normal-fat cheeses. The growing demand during later years for delicious food with less fat has stimulated the development of new semihard cheese varieties with FDM of 20% or less. Specific ripening cultures may be used in such low-fat cheeses, as heat-treated lactobacilli are in the cheese varieties Kadett (20% FDM) and Västan (20 or 10% FDM, respectively) that are produced in Sweden for the past 10 years. Magré is a Swedish reduced-fat cheese (30% FDM) that is made with the addition of PAB.

II. CHEESEMAKING

Traditionally, semihard cheese is made in Scandinavia from bovine milk using calf rennet and mesophilic DL-starter (see Sec. III below). The milk is commonly collected from two to four meals, and deep cold storage is used at the farms as well as at the dairies. This treatment of the cheese milk decreases the coagulation ability that mainly is restored by pasteurization (73°C/15 sec) and sometimes, depending on season and cheese variety, also an addition of $CaCl_2$ is needed. Other bacteria than the starter bacteria may be added during cheese production. These include PAB to Jarlsberg, Grevé, Magré and Svenbo; *Lactobacillus helveticus* to low-fat cheese; and *Brevibacterium linens*, which may be sprayed on the surfaces of some Danish cheeses (Table 1).

Calf rennet is commonly used and has not easily been replaced by other coagulants. The rennet is active during the primary casein breakdown during cheese ripening, and the characteristic physical properties of the ripened cheeses are dependent on its specific activity. The main coagulating enzyme in calf rennet is chymosin. Depending on the age of the calf and on its feedstuff, the calf rennet also contains different amounts of of the proteolytic enzymes bovine pepsin and gastricsin (1). Chymosin is an aspartic proteinase, which has its activity optimum at low pH (pH 5.0 with α_{s1}-casein as substrate) and a preference to cleave Phe-X and Leu-X peptide bonds (2,3). Rennet-mediated milk coagulation begins with a specific cleavage of the κ-casein bond Phe_{105}-Met_{106} to release the hydrophilic glucomacropeptide, which is lost in the whey. Structural rearrangement of the remaining hydrophobic para-casein effect coagulation and later syneresis when the whey is expelled from the cheese grains.

The temperature/time periods used during production of the semihard cheeses differ among the cheese varieties within certain limits as exemplified in Fig. 1 for a cheese in the lower part of the moisture content interval (Table 1). Coagulation temperature may be increased by one or two degrees for low-fat cheeses and stirring temperatures may be as high as 35°C. Lower production temperatures and shorter holding times are used to obtain the higher moisture content of some semihard cheese varieties. A variation of temperature/time periods are used to obtain different moisture as well as mineral contents, but they also influence the starter bacteria and cause variations in their further development and contribution to cheese ripening.

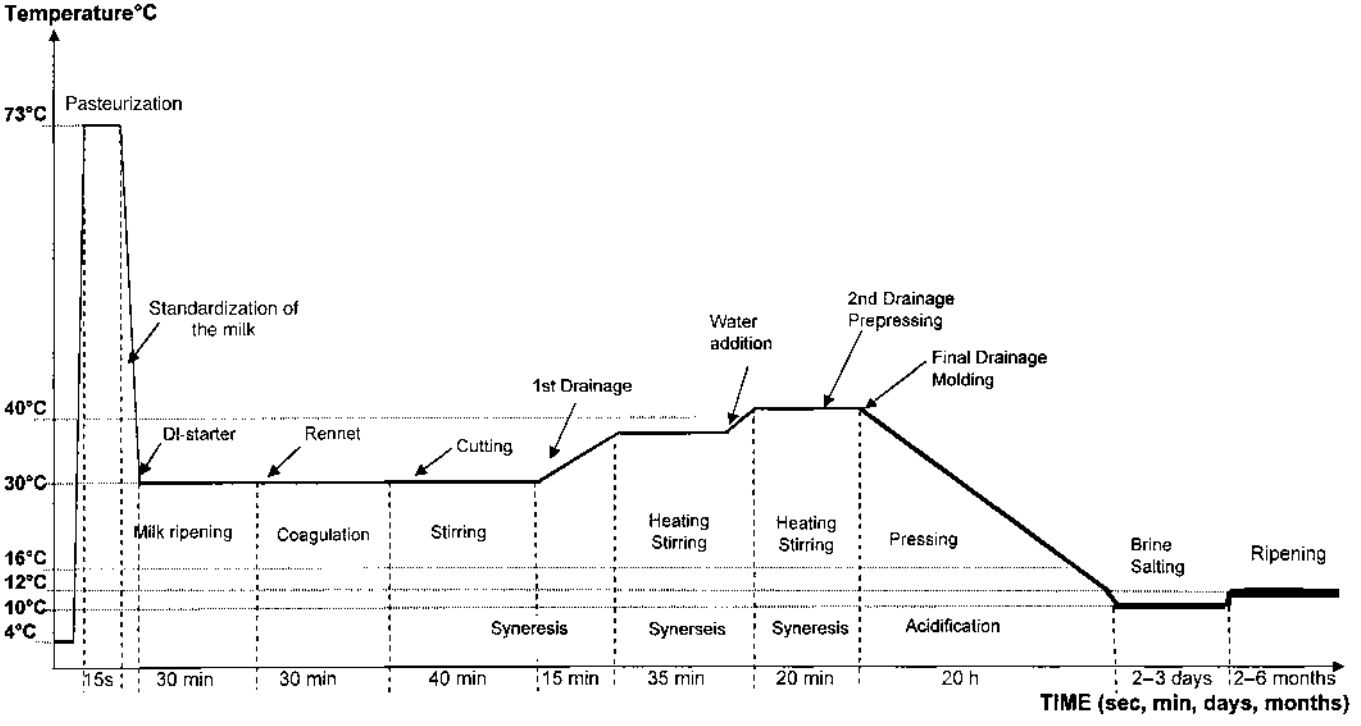

Figure 1 Typical pattern of time/temperature intervals for the production of semihard cheese in the lower moisture content interval, such as Herrgård cheese (Table 1).

Traditionally some Scandinavian cheese varities were made with higher cooking temperature than today, and specific characteristics of Herrgård still depend on the combination of using a mesophilic starter culture in combination with longer time at somewhat higher cooking temperatures than those cultures commonly are used for. This technique using mesophilic starters and very long holding times is further developed for production of the hard Swedish cheese Västerbotten. This cheese has a characteristic hard texture that differs from most other hard cheeses that are made with thermophilic starter and higher cooking temperatures.

Semihard cheese with round eyes is prepressed under whey, whereas the whey is separated from the grains before the pressing of cheese with an open texture. Acidification takes place in cheese mainly after molding during the first 24 hr while the lactose is almost completely converted into lactic acid. To prevent pH from decreasing too much, parts of the whey may be removed and water is added to the vat. The minimum pH of the semihard cheese varieties is commonly around pH 5.1–5.3 and depends on cheese variety. DL-starter bacteria in semihard cheese have preferentially used up the citrate after one or two weeks, while CO_2 is produced leading to the eye formation.

The semihard cheeses are brine salted and kept within a NaCl solution of about 20–22% for different periods of time depending on cheese variety. Small-sized, low-fat, high-moisture cheeses may be salted for as short a time as 8 hr. dryer cheeses may be kept in the brine for up to 3 days to achieve a salt content of about 1–2% in cheese. The mild, high-moisture, and smear surface-ripened cheese varieties need only a couple of weeks of ripening, whereas several of the other semihard cheese varieties are ripened from 3 months and up, to a year or more.

III. MESOPHILIC DL-STARTER CULTURE

Mesophilic undefined starters with mixed species are used in the production of semihard cheese. This kind of starter is composed of several strains of lactic acid bacteria that give rise to the development of a dynamic population during the manufacture and ripening of cheeses. They are referred to as DL-starters because of their composition. The bacterial strains are of four different kinds; namely, the two main acid producers, *Lactococcus lactis* subsp *cremoris* (*Lc. cremoris*) and *Lc. lactis* subsp. *lactis* (citrate$^-$) (*Lc. lactis*) and those metabolizing citrate, *Lc. lactis* subsp. *lactis* (citrate$^+$) (*Lc. diacetylactis*) (D) and *Leuconostoc mesenteroides* subsp. *crermoris* (*Leu. cremoris*) (L). The citrate-using bacteria (*Lc. diacetylactis* and *Leu. cremoris*) are responsible for eye formation by production of CO_2 from citrate with simultanous production of flavor compounds such as diacetyl. The three different kinds of *Lc. lactis* strains have historically been considered to belong to different species (i.e., *Streptococcus cremoris*, *S. lactis*, and *S. diacetylactis*). Improved methods for genetic comparison have later shown that the bacteria are very closely related, that *Lc. cremoris* and *Lc. lactis* are of the same species, and that *Lc. diacetylactis* is a biovariant of *Lc. lactis*. The only difference between *Lc. diacetylactis* and *Lc. lactis* observed consistently is a plasmid in *Lc. diacetylactis* coding for citrate permease and lyase, making them able to transport citrate into the cell where it may be converted to diacetyl. Today *Lc. lactis* and *Lc. diacetylactis* are referred to as *Lc. lactis* subsp. *lactis* (citrate$^-$) and *Lc. lactis* subsp. *lactis* (citrate$^+$). Some important properties of the four kinds of starter bacteria are given in Table 2 (4).

Commonly *Lc. cremoris* and *Lc. lactis* make up about 80–90% of the bacteria found in the starter. The development of the different groups of bacteria depend on the manu-

Table 2 Some Characteristics of the Bacteria Found in Mesophilic, Undefined DL-Starter

Organism	Sugar fermentation	Enantiomer of lactate	Fermentation of glu	gal	lac	Citrate metabolism	NH_3 from arginine	Growth at 40 °C
Lc. cremoris	EMG	L+	+	+	+	–	–	–
Lc. lactis	EMG	L+	+	+	+	–	+	+
Lc. diacetylactis	EMG	L+	+	+	+	+	+/–	+/–
Leuc. cremoris	PKP	D–	+	+	+	+	–	–

glu, glucose; gal, galactose; lac, lactose; EMG, Embden-Meyerhof glycolytic pathway; PKP, phosphoketolase pathway
Lc. cremoris, *Lactococcus lactis* subsp. *cremoris*
Lc. lactis, *Lc. lactis* subsp. *lactis* (citrate$^-$)
Lc. diacetylactis, *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*
Leuc. cremoris, *Leuconostoc mesenteroides* subsp. *cremoris*

facturing protocol for the different cheese varieties and are exemplified by comparing normal-fat Herrgård 45% with relatively high cooking temperatures and long cooking time and low-fat Danbo 20%, for which both lower temperatures and shorter time periods are used (Fig. 2). Typically the acidifiers (*Lc. cremoris* and *Lc. lactis*) grow to a higher number in cheeses with lower cooking temperatures, which are closer to the optimal temperatures for these bacteria (5). In the semihard cheeses made using the lower temperatures also, *Lc. cremoris* has an advantage over *Lc. lactis* which will be more important in those made at higher temperatures (6). In cheeses where the growth of *Lc. diacetylactis* is limited—for instance, because of the cooking temperature—the *Leuconostoc* that grow at lower pH than *Lactococcus* may develop to a higher number in cheese because there is more citrate left. The normal variation of milk influences the starter population—for example, less manganese in milk during winter is unfavorable to *Leuconostoc* (7).

The undefined DL-starters contain an unknown number of strains of bacteria. During growth, these strains react in different ways to environmental changes such as variations in milk and cheese composition, pH, and temperature. Many properties of importance for cheese manufacture vary among the bacterial strains, such as growth rate, sensitivity to temperatures and to autolysis, phage tolerance, and the proteolytic and peptidolytic activities as well as other metabolic activities. Important factors influencing the dynamics of the DL-starter population have been reviewed (6).

The number of bacteria and the ratio between the different starter strains in fresh cheese will certainly influence the ripening process. The starter bacteria do not multiply to larger numbers than they have reached already after a day or two, when the lactose is exhausted. After that, the numbers of the different bacteria decrease or are maintained at a certain level. Viable *Lc. diacetylactis* have been found at constant levels (10^7–10^8/g) over a period of time for several months in semihard cheese, whereas the total number of *Lc. lactis* and *Lc. cremoris* decreases rapidly to 1% or less within a couple of weeks. Leakage of enzymes, autolysis of bacterial cells, and possibility for intracellular enzymes to act directly in the cheese matrix are important parts of the ripening process. The bacteria exist in many different physiological states in cheese, and a decreasing number of starter bacteria found in cheese is no guarantee that free enzymes are active in the cheese matrix (5,8). The existence of bacterial spheroplasts in cheese have been shown by electron microscopy (9) and evidence has been given for strain dependence of enzyme release from spheroplasts (10). A large variation in autolytic behavior has been found among single strains of starter *Lactococcus* under conditions of starvation in phosphate buffers (11).

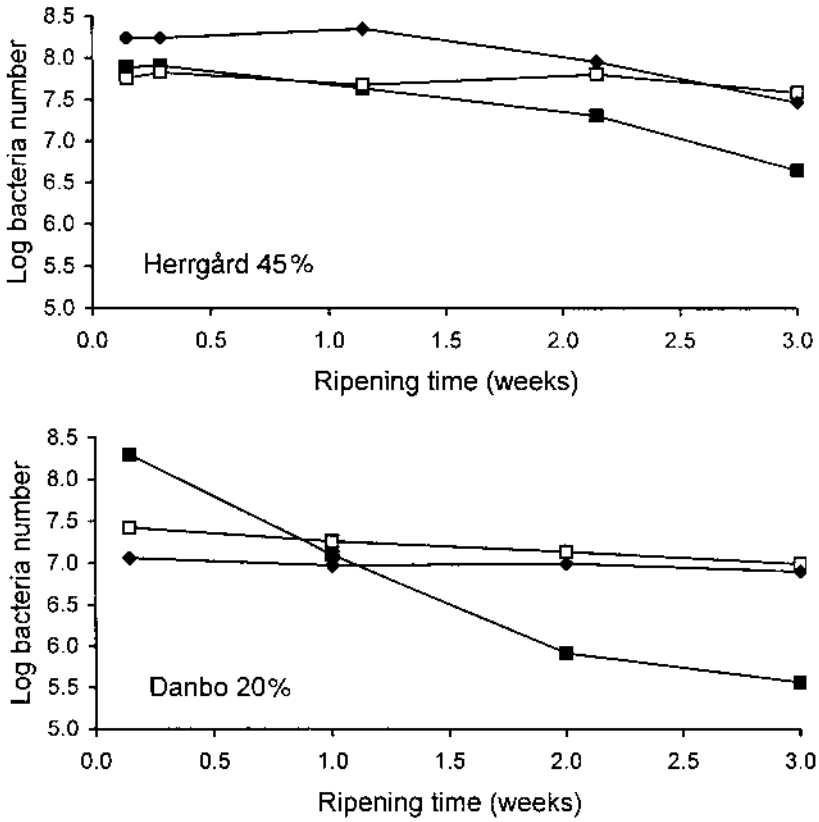

Figure 2 Development of mesophilic DL-starter in normal-fat (45% FDM) Herrgård and low-fat (20% FDM) Danbo cheese. Filled squares, *Lc. lactis* subsp. *cremoris* and subsp. *lactis* (citrate$^-$); open squares, *Lc. lactis* subsp. *lactis* (citrate$^+$); filled diamonds, *Leutonostoc*. The bacteria were analyzed using the Leesment method. (From Refs. 40 and 41).

IV. CHEESE-RIPENING MICROFLORA

A. Nonstarter Lactic Acid Bacteria (NSLAB)—*Lactobacillus paracasei*

There are almost always bacteria that are not added as a culture during cheese production that grow to high numbers in the cheese during ripening. Facultative heterofermentative *Lactobacillus* (FHL) and most commonly *Lb. paracasei* are found dominating in high-quality Scandinavian semihard cheeses, and their importance increases with ripening time (12–14). They are present at low numbers at the beginning of ripening, and no significant influence on the primary production of medium-sized peptides from casein in cheese has been shown or indicated. They may have caseinolytic enzymes, but they do probably not need them in ripening cheese with its large supply of small peptides and amino acids produced by the starter bacteria. They are extremely good survivors in a nutritional restricted environment that is changing slowly all through ripening, and one could expect them to have strict regulation mechanisms that allow production of enzyme systems only when they are needed. Their role in ripening of the semihard cheese varieties is under investigation. It is obvious that their importance is largest in long-time-ripened cheeses and they may interact

with the starter in characteistic ways. The FHL occupy a niche in the cheese and prevent detrimental bacteria from growing and metabolizing. This may result from plain competition for nutrients, but also direct antimicrobial activities could be used. Defined strains of these bacteria, mainly *Lactobacillus paracasei*, may also be added as adjuncts during cheese production. Flavor production in the Swedish cheeses Hushållsost, Svecia, Präst and Herrgård and the Norwegian cheese Norviega depends completely on DL-starter bacteria and the nonstarter FHL.

B. Smear Surface Microflora

The smear surface-ripened Danish cheeses may be sprayed by *Brevibacterium linens* or just treated in a way that stimulates the growth on the cheese surfaces if the right bacteria already are present at significantly large numbers in the salting or ripening room. Usually, yeasts establish first on the surfaces and effect an increase in pH to more suitable levels for *Br. linens*. The smear surface microflora contain several kinds of bacteria that contribute to lipolysis, to a strong proteolysis at the surface, and to production of sulfuric aroma compounds by catabolism of sulfuric amino acids (15). The microflora growing on the surface of cheese may contribute with some alternative biochemical activities because it grows aerobically. Oxidative deamination can be used in amino acid catabolism, and this activity produces ammonia that will easily migrate into the cheese body, increase pH, and stimulate the activity of proteolytic enzymes such as plasmin and the lactococcal cell-envelope protease lactocepin. Esrom is a smear surface–ripened cheese as are most brands of Danbo and Havarti cheeses (Table 1).

C. Propionic Acid Bacteria (PAB)

Cultures of propionic acid bacteria (PAB) are added to Jarlsberg, Grevé, Magré, and Svenbo (Table 1), giving these cheeses their characteristic flavor and texture. To stimulate the growth and activities of PAB, the pH of the cheeses is somewhat increased, the salt content is decreased, and the cheeses are ripened fo a period of a couple of weeks at a higher temperature (14–18°C). PAB contribute to CO_2 production, and those cheeses typically have very large eyes, as a result of gas production from both DL starter and PAB. CO_2 is produced together with propionic and acetic acid from lactic acid, and PAB also contribute to producing flavor compounds. Their proteolytic enzyme system is mainly intracellular and contains specific peptidases releasing phenylalanine and proline (16). Proline contributes to the sweet note of PAB cheeses.

D. Adjuncts and Heat-Treated Cells of *Lactobacillus helveticus*

Low-fat, semihard cheese varieties, Kadett (20% FDM), Västan (20% or 10% FDM) (Table 1), and some types of Samsø (30% FDM) are produced with adjuncts of *Lb. helveticus*. A spray-dried powder of heat-treated cells is used in Kadett and Västan to accelerate the peptide breakdown in those cheeses (17). The heat-treated cells contain a large amount of highly active intracellular peptidases with broad specificity, and the activity of the cell-bound protease is reduced and the acidification ability is inactivated by the heat treatment. The peptidases are kept within their own cell membranes in the cheese vat and will be incorporated into the curd. The intracellular peptidases are released early in cheese and influence the ripening process. The amount of amino acids produced within some weeks is far larger than in any normal-fat cheese and both flavor and consistency are improved.

Compared to a control cheese without heat-treated *Lb. helveticus*, the consistency is shorter, more ripened and the flavor is mild, clean, nutty, and aromatic, with a sweet note.

V. CHEESE RIPENING

The main pathways of cheese ripening in the Scandinavian cheese varieties are similar to those in other semihard cheese varieties (18). Lactose, citrate, milk fat, and casein are hydrolyzed during ripening while texture is developed, eyes are formed, and flavor compounds are produced. Lactose is rapidly converted into lactic acid and should not be present in the cheese after a couple of days. When pH has reached its minimum after one or two days, it will increase slowly during ripening mainly due to proteolysis and faster in cheese ripened with a smear surface microflora producing ammonia (Fig. 3).

Citrate is converted to CO_2, diacetyl, acetoin, and butanediol by the DL starter bacteria and should be exhausted after a week or two. The eye formation in semihard cheese is dependent on these activities, and in those varieties that contain propionic acid bacteria, even larger eyes are produced.

With the exception of cheese varieties ripened with a surface microflora, lipolysis is limitd in semihard cheese varieties. However, volatile compounds that may contribute to cheese flavor are produced in significant amounts from fat hydrolysis (fatty acids, β-keto-acids, methylketones, lactones). Cheese texture and flavor depend on proteolysis, which is described in more detail below.

A. Primary Proteolysis

Commonly, two mechanisms dominate the primary hydrolysis of casein of semihard cheese, and that is plasmin activity on β-casein at the three sites lys_{28} - lys_{29}, lys_{105} - his_{106}, and lys_{107} - glu_{108} and chymosin activity on α_{s1}-casein at the site phe_{23} - phe_{24}. The large casein fragments produced are not soluble at pH 4.6 (the isoelectric point of casein). They are successfully analyzed by capillary electrophoresis (CE)—the γ-caseins that are equivalent to β-casein (29-209; 106-209; 108-209) and α_{s1}-I-casein that is α_{s1}-casein (24-199). Danbo

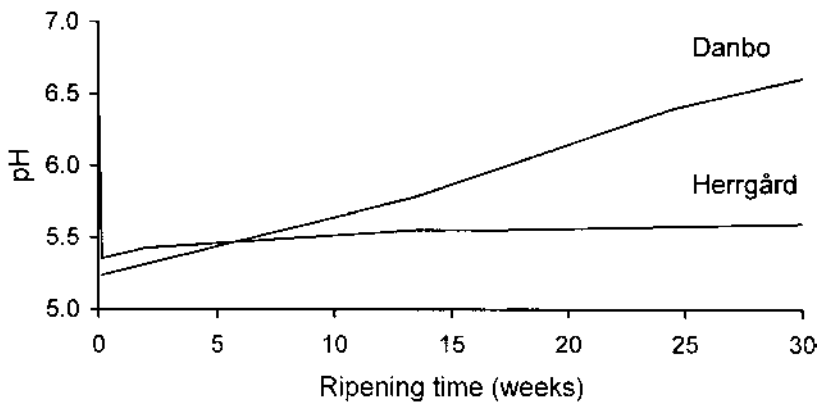

Figure 3 Typical development of pH during ripening of semihard cheese varieties. The Herrgård cheese was ripened with waxed surfaces; the Danbo cheese with a smear surface microflora producing ammonia.

20% has a relatively large content of moisture in fat-free substance (MNFS), and results of both plasmin and chymosin activity is clearly seen already after 24 hr (Fig. 4). The breakdown products of chymosin activity on α_{s1}-casein are not seen in the dryer Herrgård cheese at this early stage of ripening, whereas plasmin is quite active already during cheese production (18). It may be a result of the higher production temperatures used for Herrgård, which partly inactivate chymosin while the activating plasminogen to plasmin is stimulated.

The main calf rennet coagulating enzyme, chymosin, may cleave β-casein at leu_{192} - tyr_{193}; however, in cheese this is only possible with some special conditions fulfilled, among them a low salt content as in the interior of brine-salted cheeses during the first weeks of ripening (19). If it is produced, this peptide should be further broken down during ripening. The corresponding β-casein fragment (f1-192) has not been identified by analyzing semihard cheese for casein components using CE, and neither have its further breakdown products from plasmin activity.

B. Starter *Lactococcus* Protease and Peptidase Activities in Cheese

The starter bacteria of the genus *Lactococcus* have a well-characterized cell envelope–associated protease (CEP) that has been named PrtP and lactocepin (20,21). It hydrolyzes β–casein in milk and case in solution, and some of its genetic variants also hydrolyze α_{s1}-casein. In cheese, however, mainly activity on the chymosin- and plasmin-produced peptides have been shown (22). Especially, results of lactocepin activity on the peptides α_{s1}-casein (1-23) and β-casein (1-28; 29-107) have been shown in cheese (23,24).

The starter lactococci have a well characterized system of intracellular peptidase enzymes by which they are able to digest the peptides and release all individual amino acids of casein. This system is well known both at a biochemical and at a genetic level (21,25). Several experiments have been made over a long time showing that autolysis increases

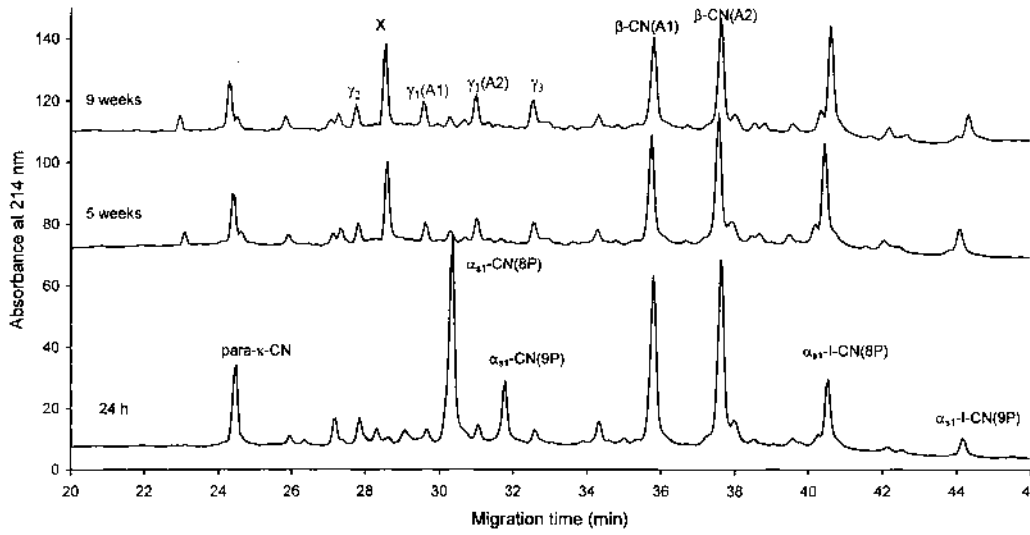

Figure 4 Casein components in low-fat (20% FDM) Danbo cheese analyzed with capillary electrophoresis (CE) at different ages. CN, casein; γ, γ-casein; X, unknown.

amino acid content in cheese, but the mechanisms of these activities in cheese and the role of all the different peptidases working in cooperation are not well understood. By using strains deficient for some of the peptidases, the complexity can be studied, but the results obtained from these experiments so far are not easily interpreted. Different combinations of aminopeptidases are apparently complementary to each other.

C. Nonstarter *Lactobacillus paracasei*

The *Lb. paracasei* have a complex system of peptidases (21,25,26) and may be supplementary to the starter-mediated release of amino acids. The specificity of the peptidases vary, and some *Lactobacillus* strains are for instance more active on releasing proline than the starter and these may dominate in semihard cheeses with a sweet note (27). The *Lactobacillus* strains in cheese may also be complementary to the starter bacteria by producing special flavors using a different equipment of enzymes for amino acid catabolism.

VI. AMINO ACID METABOLISM

Lactic acid bacteria release amino acids from casein in cheese for the purpose of using them in protein synthesis, cell metabolism, and maybe as energy sources (27). Their enzyme systems for amino acid metabolism are not as well known as those for amino acid release, but some recent results are available from the research on amino acid catabolism and production of cheese flavor compounds (Table 3) (25,28). An important first step in this catabolism of cheese bacteria is aminotransferase activity that oxidizes an amino acid to its corresponding α-keto acid by moving the amino group to another α-keto acid that simultaneously is reduced to its corresponding amino acids. All aminotransferases described so far in LAB use mainly α-ketoglutaric acid as amino group acceptor and produce glutamic acid. There are, however, at least two more possibilities that are described in higher organisms: pyruvate to alanine and oxaloacetic acid to aspartic acid. Nitrogen-fixation bacteria

Table 3 Amino Acid Metabolism in Semihard Cheese Varieties Made from Bovine Milk Using Calf Rennet and Mesophilic DL-Starter in Scandinavia

Amino acids	Reaction	Origin of activity
Arg	Deimination to Cit and Orn	Starter (NSLAB)
Lys, Pro	Accumulate (aminopeptidases in matrix)	Starter, adjunct, NSLAB
Leu (Ile, Val)[a]	Catabolized to aroma compounds	Starter, adjunct, NSLAB
Phe (Tyr, Trp)[a]	Catabolized to aroma compounds	Starter, adjunct, NSLAB
Met	Catabolized to aroma compounds	Smeared surface bacteria
Ser	Dehydrative deamination	NSLAB
Glu, (Asp, Ala)[a]	Amino group carriers	Starter, adjunct, NSLAB
Glu	Decarboxylation to GABA[b]	Contaminants
Tyr, His, (Trp, Orn, Lys, Phe)[a]	Decarboxylation to biogenic amines and CO_2	Contaminants

[a] Amino acids within brackets are possible, but commonly less metabolized in the semihard cheese varieties in question.
[b] GABA, γ-amino butyric acid.

use aspartic acid to make aspargine during anaerobic conditions and that could be possible in cheese, as well—similarly, glutamic acid to glutamine. Flavor has been enhanced in cheese by addition of α-keto glutaric acid up to a limit above which other enzyme activities of the pathways leading to aroma compounds became the limiting factors (29,30). In the semihard cheeses, the sulfuric breakdown products from methionine are mainly important in those ripened with smear surface microflora. Breakdown of especially leucine but also phenylalanine has been shown to be important and increases during ripening of the semihard cheese varieties in Scandinavia (27). Cheese aroma compounds are produced from breakdown of those two amino acids.

Decarboxylation is a well-known activity by which bacteria obtain an increase in their intracellular pH when the environment becomes too acid. The ability varies with species and strains, and in mesophilic starter cultures those strains are avoided that may produce gas and biogenic amines, such as tyramine and histamine by decarboxylation of amino acids at cheese pH. Some *Lc. lactis* subsp. *lactis* strains have been isolated from mesophilic starters that are able to decarboxylate glutamic acid to GABA (γ-aminobutyric acid) and CO_2 (31). Fortunately, the *Lc. lactis* strains investigated so far only decarboxylate glutamic acid after being exposed for several hours to pH values that are lower than is normal for cheese (27).

The significance of bacterial use of amino acids as energy sources in cheese is not clear. Some starter bacteria (e.g., *Lactococcus lactis* subsp. *lactis*) convert arginine to ornithine and may produce ATP (adenosine triphosphate) this way, and ornithine is actually produced in cheese with these bacteria present (18,27). In those cheeses, commonly also citrulline is found; and because it is an intermediate between arginine and ornithine, its presence could indicate uncompleted digestion because of lack of components needed for further metabolism, or it could be a result of autolysis of starter bacteria. In some cheeses NSLAB may also contribute to the conversion of arginine to citrulline and ornithine, especially if the starter bacteria do not effectively use up all the arginine that is released during ripening. Serine is used by nonstarter lactobacilli and by added cultures of *Lb. plantarum* (32,33). Serine may be dehydratively deaminated in cheese and further converted to pyruvate, which is a potential energy source.

VII. AUTOLYSIS

The role of autolysis in ripening of semihard cheese varieties has been the subject of lively discussion and we still lack the knowledge to understand it very well. There are no doubts that autolysis and release of intracellular enzymes is important to hydrolysis of mainly smaller peptides and amino acid release (17, 34–38). Autolysis of one strain each of *Lc. lactis* and *Lc. cremoris* was evaluated in a semihard French cheese of Saint-Paulin type (39). The investigators measured bacterial cell viability and studied the morphological changes and leakage of peptidases. The *Lc. cremoris* bacteria had already begun to autolyze during the first week of cheese ripening. In those cheeses, bacterial cells in three states of lysis were distinguished by electron microscopy: spheroplasts with minor leakage (15% of the cells), spheroplasts with rupture of the cytoplasmic membrane (55%), and almost completely lysed cells (25%). The cells of the *Lc. lactis* strain retained their viability for a long time; only some spheroplasts were found, and they had no rupture on the cell membrane. Increased amounts of peptidases as well as small peptides and amino acids were found in cheeses with the more auotolytic strain, and these cheeses were less bitter than the others.

The influence of autolysis on amino acid catabolism and flavor development in cheese is less clear, though. Theoretically, individual enzyme steps may be facilitated, but whole

chains of biochemical events needed for some flavor compound formation may become less efficient after autolysis. The optimal performance of growth, metabolism, and autolysis of different bacteria is likely to be dependent on cheese variety.

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16

Cheeses Made with Thermophilic Lactic Starters

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I. CHEESES INVOLVING THERMOPHILIC LACTIC STARTERS: FROM ROMAN TIMES TO NEW PRODUCTS

More than 9.5 millions tons of cheeses are produced per year over the world. Among them, cheeses involving thermophilic lactic starters, either alone or in combination with other starters, are widespread. The most largely produced are Swiss-type cheeses and pasta filata (stretched curd cheeses), with their respective "archetypes"—Gruyère / Emmental and mozzarella (Table 1). The origin of Gruyère-type cheese is still uncertain. Medieval manuscripts already described the production of these kinds of cheeses. In the 13th century, highlanders from Franche-Comté and Switzerland produced large (about 30 kg) cooked cheeses as a way to keep the milk produced by grazing cows during summer. The term Gruyere appeared formally in the 17th century. Then variations occurred in the technology or ripening conditions, leading to more or less heavier cheeses (60 to 130 kg), such as Beaufort and Comté, and then Emmental with big eyes in the 19th century. The origin of pasta filata cheeses is also very ancient: the consumption of provolone (mostly smoked and well matured) is described in Roman times (1).

All these cheeses require as starters thermophilic lactic acid bacteria (LAB), because the production process requires an incubation of the curd at relatively high temperatures (above 45°C). Among LAB, three species are able to grow and to acidify at such temperatures: *Streptococcus thermophilus* and two lactobacilli species, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* (with two subspecies: *L. delbrueckii* subsp. *lactis* and *L. delbrueckii* subsp. *bulgaricus*). They can be used alone but are most often used together, in a defined ratio of streptococci/lactobacilli. Indeed, the well-known symbiosis phenomenon between these species leads to better growth and acidification. However, strain-to-strain interactions can occur, and the choice of which pairs of *Streptococcus thermophilus* and *Lactobacillus* sp. strains to be used as acidifying starter must be carefully made.

In pasta filata and several hard-cooked cheeses such s Grana or Beaufort, thermophilic starters are used alone, transforming lactose or galactose into lactate. In cheese varieties like Emmental and some other so-called Swiss-type cheeses (e.g., Jarslberg) thermophilic LAB are associated with dairy propionibacteria, which transform the lactate produced into propionate and acetate (both major flavor compounds in Swiss cheeses) and into CO_2

Table 1 Cheeses Involving Thermophilic Lactic Acid Bacteria (not exhaustive list)

Hard-cooked cheeses	
Emmental, Comté, Gruyère, Hartkäse, Berg-Alpkäse, Jarslberg Abondance, Pecorino Romano	*S. thermophilus* and *L. delbrueckii* subsp. *lactis* or *L. helveticus* or both lactobacilli
Beaufort, Grana, Parmigiano, Asiago, Sbrinz	*L. delbrueckii* subsp. *lactis* and/or *L. helveticus*
Pasta filata or stretched-curd cheeses	
Mozzarella, Pizza cheese, Provolone	*S. thermophilus* and eventually *L. delbrueckii* subsp. *bulgaricus* or *L. helveticus*
Soft cheeses	
Camembert & Brie (stabilized), Reblochon, St Nectaire, Livarot, Pont-l'Evêque, Crescenza	*S. thermophilus*
Blue cheeses	
Gorgonzola, Bleu de Bresse, Bleu de Gex, Fourme d'Ambert	*S. thermophilus* and *L. delbrueckii* or *L. helveticus*
Other cheeses	
Feta, White brine cheeses	*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*
Cheddar	*S. thermophilus*

(essential for the cheese opening). Interactions with this flora are of the utmost importance in determining the final quality of these types of cheeses, and the efficiency of the complete ecosystem, thermophilic LAB with propionibacteria, must be controlled.

More recently, thermophilic LAB are being used as starters in technologies without cooking of the curd, such as some blue cheeses or soft cheese varieties (Table 1). The purpose of such a use is to achieve rapid acidification (at about 38°C) to a pH of 5.2; the so-obtained fast drainage leads to a less demineralized cheese with generally a softened and homogeneous texture. In this case, interactions can take place with mesophilic LAB if they are used concomitantly, and with surface flora like yeasts and fungi (*Penicillium roqueforti* and *Penicillium camemberti*).

Finally, thermophilic LAB, most often thermophilic lactobacilli, are also used as adjuncts in cheeses in other families such as hard cheeses, soft cheeses, UF-cheeses, and low-fat varieties, in which their main role is not acidification; instead, they are expected to improve either the flavor or the final texture through their enzymatic activities (i.e., peptidases).

Despite these wide and varied uses for thermophilic LAB starters in the cheese industry (Table 1), current knowledge regarding their physiology and genetics is far less than that accumulated for the mesophilic LAB, *Lactococcus lactis*. This review will start with a description of the making of hard-cooked cheeses and pasta filata. The main microbial properties of thermophilic LAB and the state of the art regarding important technological traits, such as acidification and proteolytic activity, will be summarized. Finally, the complex interactions between thermophilic LAB and dairy propionibacteria in making Swiss cheeses will be detailed.

II. TECHNOLOGY OF CHEESES INVOLVING THERMOPHILIC LACTIC ACID BACTERIA

As stated above, hard-cooked Swiss cheese like Emmental and pasta filata are widely produced worldwide: about 480,000 tons and 2 million tons, respectively. The processes for these two types are presented in Fig. 1a,b. A large part of the process is carried out at a temperature higher than 35°C.

A. Hard-Cooked Cheeses

After facultative heat treament (thermization), the composition of the milk is standardized (casein to fat ratio) and cold maturation is achieved through the addition of mesophilic lactic starters (mostly lactococci mixed starter) and a 15 hr incubation at 13°C. The milk is then heated to 32°C and inoculated with thermophilic starters with a defined ratio of *S. thermophilus* to lactobacilli. In the case of Swiss cheese (2) dairy propionibacteria (especially *Propionibacterium freudenreichii*) can also be inoculated as starter. After coagulation, cutting, and stirring, the curd grains are heated at least 35 min at 52°C and then drained under vaccum and pressed. The transformation of lactose in lactic acid by thermophilic starters occurs mainly during pressing, leading to a pH of 5.2–5.4 (Fig. 1a). The cheeses are then ripened in various conditions of temperature depending on whether the propionic acid fermentation should be enhanced (Emmental), limited (Comté), or avoided (Beaufort) [for details see (3)].

B. Pasta Filata

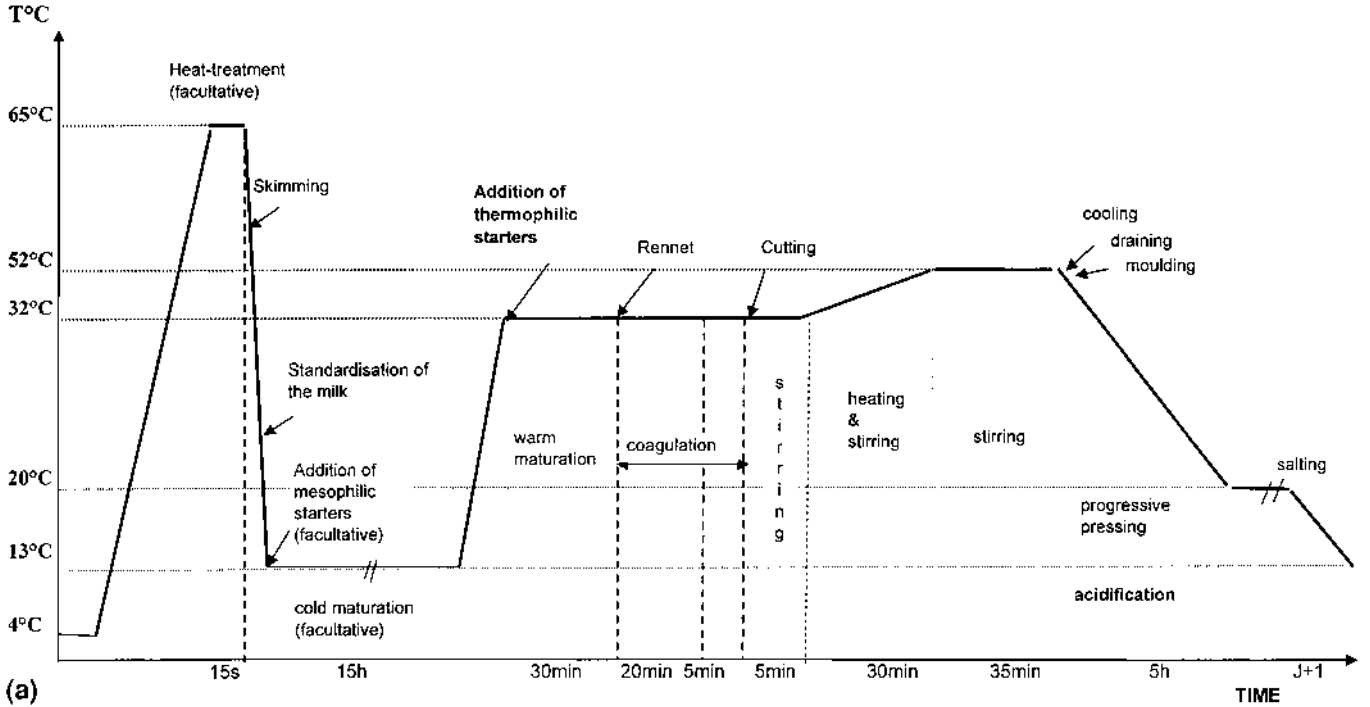
The technology of pasta filata cheeses includes a unique thermizing and texturing step at the end of the cheesemaking, just after the acidification phase (Fig. 1b). The acidified curd is dipped in hot (>65°C) whey or hot water or even salt brine, stretched mechanically, and then molded into the desired form (4). The draining pH is a decisive parameter because it governs the calcium/protein ratio in the final curd, which will determine the subsequent functional properties. The right stretching transforms the amorphous protein matrix into a network of parallel fibers, and this step has a major impact on the microbial and biochemical properties of the cheese during the ripening.

III. PROPERTIES OF THERMOPHILIC LACTIC ACID BACTERIA

A. General Microbial Characteristics

1. Growth

Thermophilic LAB species are gram-positive, microaerophilic, nonmotile, catalase-negative bacteria that share the same ecological niche: dairy environments (5,6). Their main microbial characteristics are summarized in Table 2. The species cited are obligately homofermentative, and the end product of fermentation is mainly or exclusively lactate (>90%). They are similarly auxotrophic for several vitamins and amino cids, such as branched amino acids, as well as histidine and glutamic acid, and all need rich and complex media for growth. They differ, however, in their fermentation profiles, which is very limited in the case of *L. delbrueckii* subsp. *bulgaricus* (Table 2). *Streptococcus thermophilus*


T°C
65°C
52°C
32°C
20°C
13°C
4°C
Heat-treatment
(facultative)
Skimming
Addition of
thermophilic
starters
Rennet
Cutting
cooling
draining
moulding
Standardisation of
the milk
Addition of
mesophilic
starters
(facultative)
warm
maturation
coagulation
stirring
heating
&
stirring
stirring
salting
progressive
pressing
acidification
cold maturation
(facultative)
15s
15h
30min
20min
5min
5min
30min
35min
5h
J+1
TIME
(a)

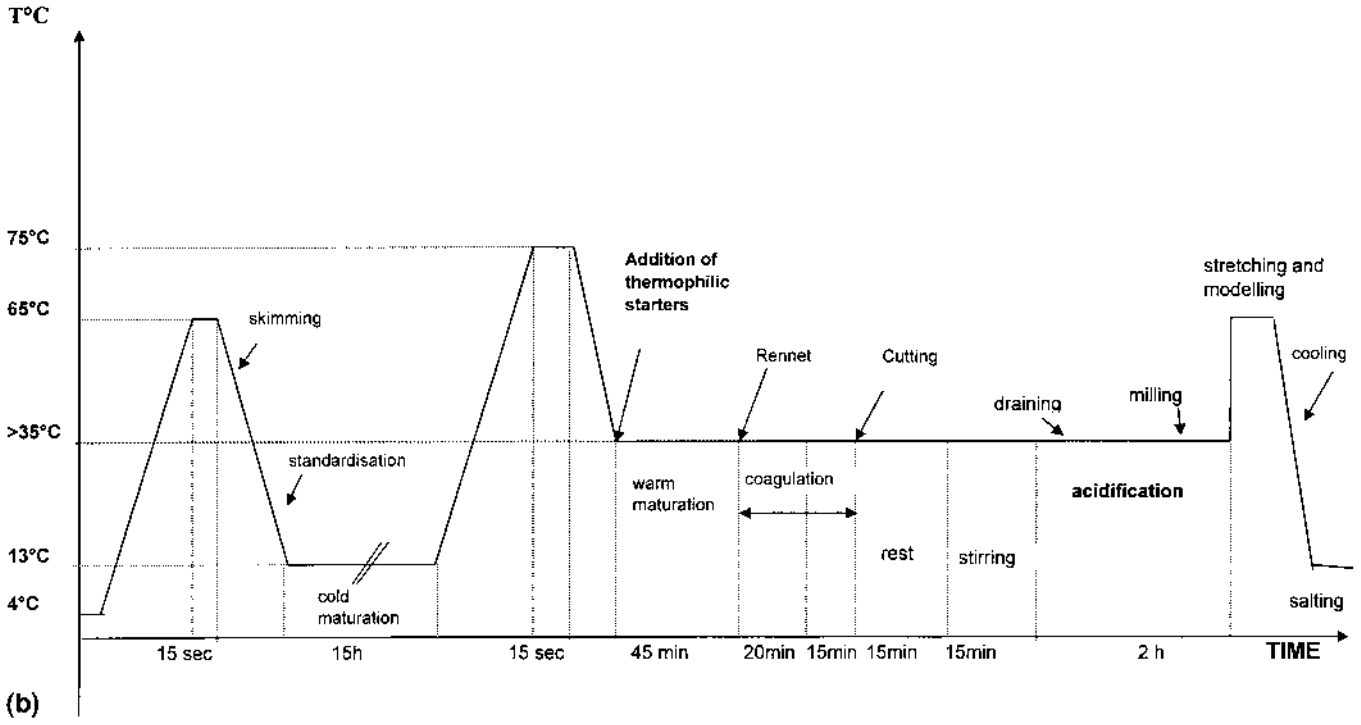


Figure 1 (a) Process of Swiss-type cheeses making (Emmental). (b) Pasta filata cheese-making process (Mozzarella). (From Garric, G., personal communication.)

Table 2 Main Characteristics of Thermophilic LAB Used for Cheese Making

		L. delbrueckii		
	S. thermophilus	subsp. *lactis*	subsp. *bulgaricus*	*L. helveticus*
Morphology	cocci (0.7–0.9 μm)	rod	rod (0.5–0.8/2–9 μm)	rod
Optimum growth T (°C)	40	38–45	38–45	38–45
Optimum pH	6.0–6.5	5.0–5.5	5.0–5.5	5.0–5.5
Generation time (min)	15–20	10–25		20–30
Lactic acid (in milk, g/L)	8–10	10–25	16–18	20–30
Isomers of lactic acid	L(+)	D(−)	D(−)	L(+) & D(−)
Lactic acid from: (* = strain dependant, % of strains + are indicated)	Glucose, lactose, saccharose, fructose* (50%), ribose* (33%)	Glucose, lactose, saccharose, fructose, maltose, mannose trehalose, galactose* (50%)	Glucose, lactose, fructose	Glucose, lactose, galactose, fructose*, maltose* (28%), mannose* trehalose* (35%)
CO_2 and NH_3 from urea	+	−	−	−

Source: Refs. 5, 6, and 75.

prefers a higher pH to grow and is the only species able to transform the urea in milk into NH_3 and CO_2.

2. Taxonomy

The position of *S. thermophilus* among other streptococci (in particular *S. salivarius*) has now been clarified. It is recognized as a separate species and specific probes have been developed (5). The taxonomic studies on the group *L. delbrueckii* led to the hypothesis that *L. delbrueckii* subsp. *lactis* is the common ancestral genotype from which two variants adapted to specialized ecological niches evolved: plants for *L. delbrueckii* subsp. *delbrueckii* and fermented milks for *L. delbrueckii* subsp. *bulgaricus*, respectively. Because of their differences in the sugar fermentation profiles, the differentiation between the three subspecies can be easily performed. On the other hand, *L. helveticus* is not phylogenetically related to the *L. delbrueckii* group (less than 40% of DNA-DNA hybridization), and several easy means of differentiation exist. For thermophilic lactobacilli, also, species-specific probes or other molecular methods were developed (7). Recently, convenient phenotypic methods based on zymogram profiles of peptidoglycan hydrolases (8) or cell wall protein profiles (by SDS PAGE) have been proposed to distinguish *L. helveticus* from other lactobacilli (9).

Various tools for strain typing have been developed for thermophilic LAB, including genomic macrorestriction profiles and random amplified polymorphic DNA (10–12) which allow collection typing and assessment of genotypic diversity.

3. Genome Data

The estimation of genome sizes (mainly by pulsed field gel electrophoresis) has produced similar values for the four thermophilic LAB discussed (i.e., 1.85–2 Mb). However the GC% of the *L. delbrueckii* group was markedly higher (49–51%) than that of *S. thermophilus* and *L. helveticus* (38–40%). Plasmids are described in the four species but their presence depends up species and strains (apparently less frequent in *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* compared to the two other species of thermophilic LAB). In only a few cases, they have been associated with specific phenotypic traits (i.e., cell wall proteolytic activity in *L. helveticus* strains, stress protein in *S. thermophilus*, antibiotic resistance). Several plasmids have been completely sequenced and characterized (13,14). The advances in elucidating the genetics of thermophilic LAB has recently been reviewed (15). Briefly, genome exchange between *S. thermophilus* and the mesophilic LAB *Lactococcus lactis* was demonstrated to occur. Indeed, some of the insertion sequences found in *S thermophilus* strains are clearly of recent lactococcal origin. Evidence for similar transfer from *L. helveticus* to *L. delbrueckii* subsp. *lactis* has also been reported. Several genes related to sugar transport, glycolysis, proteolysis, stress response, or ability to produce exopolysaccharides were cloned and sequenced (15). Most important, complete genome sequencing projects are already on going for *S. thermophilus* (J. Delcour, Belgium; A. Bolotin, France and USA) and for *L. delbrueckii* subsp. *bulgaricus* (E. Maguin, France; and by Danone). The sequence for *L. helveticus* has recently been completed (J. Steele, USA) but the data are not yet available. This complete sequencing will obviously allow significant advances in our knowledge of thermophilic LAB genetics and metabolism. However, few gene transfer methods are yet available, hampering the obtainment of mutants needed to assess the respective role of enzymes, in cheese in particular, and the potential future development of modified strains (GMO). The problem is particularly urgent for *L. delbrueckii* subsp. *bulgaricus*, for which no transformation protocol of any kind exists. In

contrast, several strains of *S. thermophilus*, including industrial strains, can be electrotransformed, and a promising method of conjugal transfer of foreign DNA was recently described for *L. helveticus* (16).

B. Technological Properties of Thermophilic Starters Contributing to Cheesemaking

Cheese-making and ripening is a complex and time-consuming process involving the gradual breakdown of milk components (carbohydrates, proteins, and fat). The major biochemical processes are acidification, proteolysis, and subsequent transformation of the released amino acids into flavor compounds, in parallel with the hydrolysis of milk fat (i.e., lipolysis). The first criteria for selection of thermophilic LAB is their ability to produce lactic acid from lactose with a defined kinetic. Indeed the rate and extent of acid production and resulting demineralization of the curd are critical determinants of cheese structure and texture.

1. Acidification

In thermophilic LAB, lactose is transported into the cell by permease systems and converted into glucose and galactose by a β-galactosidase. Glucose is metabolized through the glycolytic pathway; and galactose, when metabolized, through the Leloir pathway. Indeed, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and, depending on the strain, *L. delbrueckii* subsp. *lactis* are not able to metabolize galactose (Table 2). In this case, 1 mol of galactose is excreted in the medium per mol of lactose consumed. Although some strains of *S. thermophilus* can be galactose-positive, this property is highly unstable (the problem of residual galactose in cheese is discussed below). Depending on the species, the quantity and isomers of lactate produced may differ (Table 2). The kinetics of lactic acid production is highly strain dependent and must be tested under technological conditions, taking into account the temperature gradient for each technology (17–19). There is also considerable diversity between strains regarding temperatures at which maximum rates of acid production occured. When combined, initial acidification is due to *S. thermophilus*, which stops growth at about pH 5.2; the final acidification is essentially due to lactobacilli, because of their acid tolerance. In general, the resistance to heating of *L. delbrueckii* subsp. *lactis* strains is higher than the one of *L. helveticus* strains, explaining a later acidification with this last species (20,21). Recently, Cachon et al. (22) proposed screening LAB not only for acidification but also for reduction capacity, which can influence the oxydo reduction potential of the medium. Interestingly, acidifications with *S. thermophilus* and thermophilic lactobacilli finished before the end of the reduction phase. Oxido reduction potential creates conditions for a balanced flavor development in cheese, so this approach seems promising.

In mozzarella cheese, the impact of the rod/coccus ratio on acidification and subsequent chemical composition, proteolysis, and functional properties was determined by Yun et al. (23). Regardless of the initial ratio, cocci were dominant in the curd and resisted stretching at 57°C. The main impact on the acidification schedule is the amount of inoculation. In Swiss cheese, streptococci also grow first and reached a maximum level after 4 to 6 hr of pressing (Fig. 2). It must be underlined that acidifying properties can be unstable in some strains of thermophilic lactobacilli after successive generations, in particular in *L. helveticus* (24).

In Swiss cheeses, lactose is normally completely removed at day one at the end of pressing (pH between 5.2 and 5.5). During the ripening, pH can increase (0.2 to 0.4 units)

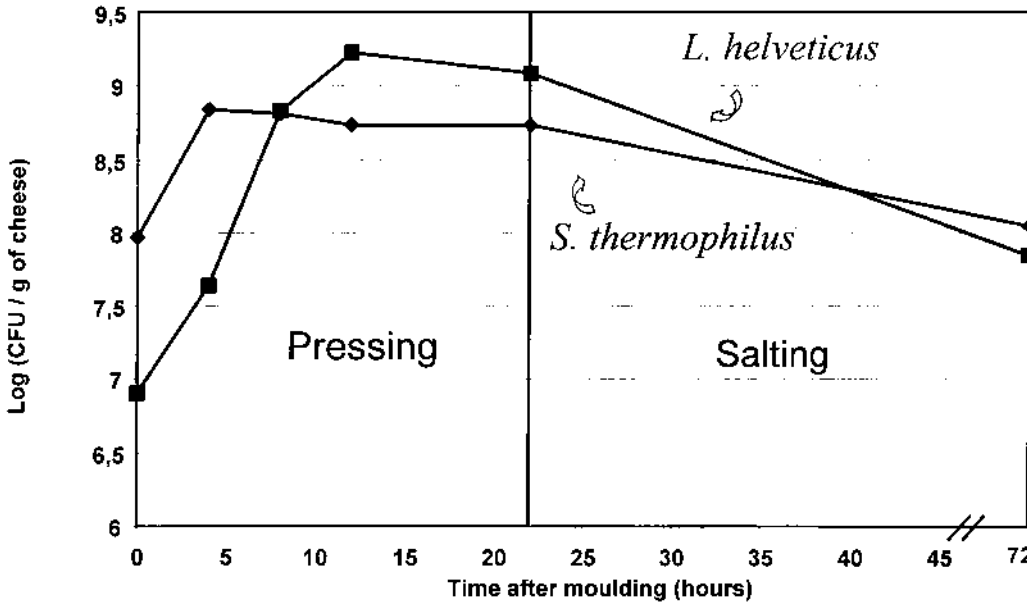


Figure 2 Population dynamics of thermophilic LAB under pressing and salting in Emmental curd. (From SM Deutsch, unpublished data.)

due to proteolysis and consumption of lactate by other flora. The absence of residual sugars is regarded as a guaranty of cheese stability during ripening. In pasta filata cheeses, variation exists. In general, the residual starter (surviving to stretching) continues to ferment lactose during the first days of ripening, resulting in negligible residual levels. By contrast galactose is fermented very slowly and can persist in pizza cheese. The level of galactose declines, of course, more rapidly when the starter includes *L. helveticus*. Thus, the starter culture plays an important role in pizza cheese browning by affecting the lactose/galactose content. In general, high level of residual galactose is regarded as a risk as it can support the growth of undesirable flora or generate post-acidification.

Phage attack can compromise dramatically the acidification step, in particular phages attacking *S. thermophilus*. In the past few years, molecular tools have allowed a better understanding of the interaction between the thermophilic LAB starter and phage populations in a cheese factory (25). However, despite the increase of knowledge about phages of thermophilic LAB (several were completely sequenced)(26,27), good hygienic practices during the preparation of the starter, rotation of strains, and use of mixed strains (in particular of *S. thermophilus*) remain the current defenses against phage attack of thermophilic LAB.

2. Proteolytic and Peptidasic Activities

Milk contains a large amount of proteins, of which 26 g/kg are caseins, but is relatively poor in free amino acids and small peptides. The presence of cell wall–associated proteases is thus essential for auxotrophic bacteria to cleave caseins into short peptides, which can then be imported and degraded intracellularly into free amino acids. Thermophilic lactobacilli have generally a strong cell wall proteolytic activity (PrtP), which is not the case with *S. thermophilus*, except in a few strains [about 3%; (28)] (Table 3). Thus, in a mixed

Table 3 Short Synthesis About Proteolytic and Peptidasic Activity of Thermophilic Starters

		L. delbrueckii		
	S. thermophilus	subsp. *lactis*	subsp. *bulgaricus*	*L. helveticus*
Cell wall proteinases				
Type	PrtS	PrtB	PrtB	PrtH, PrtY
Activity	Weak	Strong *	Strong	Strong
Binding to cell wall	Covalent	Noncovalent	Noncovalent	Noncovalent
Hydrolyze preferentially	αS1 and β casein	β casein	αS1 and β casein	αS1 and β casein
Other Observations		* inducible in milk, strongly repressed by peptides from casein		
Peptidases[a] cloned and sequenced *proline-specific peptidases	PepC, pepN, pepS, pepO	PepN, pepC, pepV, pepL, pepG, pepQ*, pepX*	PepN, pepC, pepT, pepV, pepO, pepQ*, pepX*, pepI*	PepN, pepNi, pepC, pepT, pepV, pepD, pepO, pepQ*, pepX*, pepI*, pepR, pepT

[a] pepN, general aminopeptidase; pepC, thiol amino peptidase, pepT, tripeptidase; pepV, pepD, dipeptidases; pepL, leucyl aminopeptidase; pepO, endopeptidase; pepP, aminopeptidaseP; pepQ, prolidase; pepX, X-prolyldipeptidylaminopeptidase; pepI, proline iminopeptidase; pepR, prolinase.

starter, the more proteolytic lactobacilli stimulate *S. thermophilus* growth by increasing free amino acids and small peptides, released by the PrtP activity, and that is one aspect of their symbiosis. In turn, *S. thermophilus* produces formate and CO_2, which stimulate lactobacilli growth. Several proteases of thermophilic LAB have been cloned and sequenced and all belong to the serine proteases family, with an optimum activity pH and temperature of, respectively, 6.0–6.5 and 40–45°C. However, they differed in some characteristics such as specificity, regulation, and mechanism of cell wall binding (Table 3) [for review, see (15,29–32)]. The peptides are then hydrolyzed into shorter peptides and free amino acids by numerous peptidases. Various aminopeptidases, dipeptidases, and peptidases specific to proline-containing peptides have been isolated, cloned, and sequenced (Table 3). The peptidase activity of the four thermophilic LAB species was compared using the same 34 sequenced peptides issued from β-casein by Deutsch et al. (33). Regardless of strain, *L. helveticus* was the most efficient, whereas *S. thermophilus* was not able to release free proline. This is consistent with the absence of pepIP (removing proline in a N- terminal position) and a low activity of pepQ (prolidase hydrolyzing X-pro peptides) in that species (30). Peptidases of all four species were not able to hydrolyze significantly the three phosphopeptides of the β-casein hydrolysate, providing the first experimental evidence of the intrinsic resistance of those peptides. Last, the presence of at least one carboxypeptidase was again suggested (33). As no carboxypeptidase was successfully isolated and purified, this controversial point should be clarified.

In Swiss cheese, the choice of lactobacilli has a major impact on the peptidic profiles and the final extent of proteolysis. Their cell wall proteases contribute to casein hydrolysis, in particular casein α_{s1}. However, they are especially active in the degradation of peptides resulting from rennet and plasmin activities, and in this way, they contribute to the final flavor by removing bitter peptides that are issued from the N-terminal of the α_{s1} casein or the C-terminal part of the β-casein. This ability to remove bitterness is one of the most

important traits in terms of cheese final flavor (34,35). The protease activity toward whole caseins or large peptides in cheese is largely strain and species dependent (36) and an in vitro assay was recently proposed for improving thermophilic lactobacilli selection (37,38).

In pasta filata cheeses, it was shown that the primary proteolysis was mainly due to the coagulant and that the contribution of thermophilic starters occurs in the subsequent hydrolysis of large peptides into small peptides and free amino acids. The extent of proteolysis is directly related to the ratio of rods to cocci in the starter and depends on the chosen strain of lactobacilli. The functional properties are also influenced by the starter proteolytic activity: Mozzarella cheese made with single strains of proteinase-deficient *L. delbrueckii* subsp. *bulgaricus* exhibits less browning, greater melting and less stretch than control (39).

As mentioned in the Introduction, thermophilic LAB, because of their high peptidasic activity, are also used as adjuncts in cheese to improve the flavor and or to reduce ripening time, as for example in Cheddar with a low inoculum of 10^2 or 10^3 cfu/mL (40). When added at high inoculum, the acidifying activity of thermophilic LAB cultures can be first reduced or suppressed by an "attenuation" treatment in order to avoid any effect of the adjunct on the acidification kinetics of the cheese concerned [for review, see (41)]. Of course, the attenuation treatment should not provoke any significant inactivation of the proteolytic/peptidasic activities of the strain. *Lactobacillus helveticus* is particularly valued as attenuated starter for many cheeses varieties because of its high ability to increase release of free amino acids, to reduce bitterness, and to improve flavor.

3. Lysis

In Swiss cheese, as soon as the acidification step ends, the numbers of thermophilic lactic acid bacteria decreased continuously (Fig. 2) (42). It was recently demonstrated that thermophilic starters lyse early in the curd, at least in Swiss- and Grana-type cheeses, releasing intracellular enzymes, especially peptidases, which then contribute efficiently to cheese secondary proteolysis (35,43–45). The ability to lyse was shown to be strain dependent and the mechanisms involved and the impact on cheese proteolysis have been reviewed (46). Briefly, carbon starvation (lactose or galactose) induces lysis of thermophilic starters in cheese, and the variation in extent of lysis is related to a larger free amino acid content: this emphasizes the importance of lysis ability of thermophilic LAB as a new selection criteria, as it was evidenced for lactococci.

4. Amino Acid Catabolism

The catabolism of amino acids, particularly sulfur, branched chain, and aromatic ones, can lead to important cheese flavor compounds as demonstrated for the mesophilic LAB, *Lactococcus lactis* (47,48). Few similar data are available for thermophilic LAB. Aminotransferase activity was clearly detected in cell-free extracts of *L. helveticus* by Klein and Lortal (49), and in resting cells of the three thermophilic LAB species by Helinck et al. (50). From leucine, the main aroma compounds produced by intact cells were α-ketoisocaproic acid, isovaleric acid, and 3 methylbutanol. Methane thiol was produced from methionine, but the quantities produced can vary 10-fold depending on the strain. From trytophan, the risk of producing off-flavor compounds has been emphasized (51). Thermophilic lactobacilli are also known to produce acetaldehyde in milk (by cleaving threonine by a threonine aldolase) as well as various ketones, aldehydes, and sulfur compounds. The ability to decarboxylate glutamic acid, producing CO_2, gamma-amino-butyric acid (GABA) and alpha-amino-butyric acid exists in *S. thermophilus* and may have an impact on the opening and the

taste of some cheeses (52). However, this property is highly strain dependent. The determination of the real impact of thermophilic starter on final flavor through amino acid catabolism requires further investigations, as no assay in cheeses have been published yet.

5. Esterolytic Activities

Thermophilic LAB exhibit rather low esterolytic and lipolytic activities, and very few studies have been devoted to these enzymes. Those of thermophilic lactobacilli were reviewed by Gobbetti et al. (53). The activity of *S. thermophilus* was shown to be at least twice that of mesophilic lactococci. A lipase from *L. delbrueckii* subsp. *bulgaricus* was characterized by El-Sawah et al. (54) and an aryl esterase of *L. helveticus* was recently cloned that is active in cheese conditions (55). Unfortunately, very few data are available about the contribution of thermophilic LAB in cheese lipolysis: in Swiss cheese, the role of thermophilic LAB seems minor compared to the impact of dairy propionibacteria on lipolysis (56).

6. Production of Exopolysaccharides

Thermophilic LAB produce exopolysaccharides composed mainly of glucose, galactose, rhamnose, and sometimes N-acetylglucosamine residues (57,58). This property is chromosomally encoded but is relatively unstable. Variable amounts are produced depending on the species: values from 50 to 350 mg/L and from 60 to 425 mg/L were cited for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, respectively. The EPS gene cluster was recently sequenced (15). In cheese, this production can have a major impact on texture and water content (59–61) and this has been successfully exploited to improve, for example, the texture of low-fat mozzarella. However, strains producing exopolysaccharides can also lead to draining defect and should be used carefully.

7. Conclusions

Among the technological properties of the thermophilic LAB, the most well characterized are acidification and proteolysis. Even so, however, these two essential properties cannot be fully controlled because interactions between strains and species can greatly modulate their expression in situ. The impact on ripening of their ability to produce exopolysaccharides and of their amino acid catabolism are promising fields of research and development.

IV. INTERACTIONS WITHIN THERMOPHILIC LAB AND WITH DAIRY PROPIONIBACTERIA

Numerous interactions between species can occur in cheese involving thermophilic LAB as main starters as summarized in Fig. 3. When lactococci are used for maturation, the growth and acidification by thermophilic LAB are generally stimulated during pressing. This was particularly demonstrated in Comté cheese (62); however, the mechanism is not really established. Similarly, the potential influence of the milk natural flora on the thermophilic starters growth was not really explored. Strain-specific interactions were also observed between LAB and yeasts or molds (*P. roqueforti*) in blue cheeses (63), the mold stimulations being attributed to the release by LAB of amino acids such as arginine and leucine. In Swiss cheeses, as well as in many cheeses involving thermophilic LAB, a significant growth of nonstarter LAB (mainly *L. paracasei*) has been described ($>10^8$ cfu/g at the end of the ripening). NSLAB growth, in particular of citrate positive strains, can slow down propionic cid fermentation (64). However, the most documented interactions

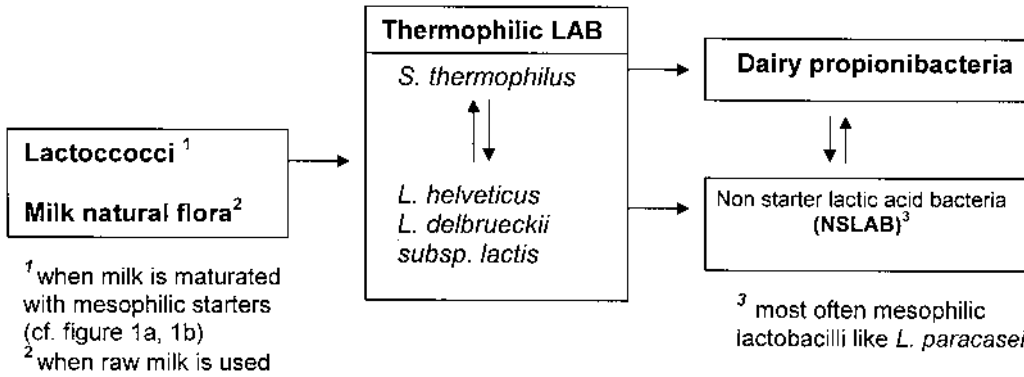


Figure 3 Interactions between sequential and concomitant populations in cheese involving thermophilic LAB.

are those observed within thermophilic LAB, and between propionibacteria and thermophilic LAB starters, as summarized below.

A. Interactions Between Thermophilic LAB

Interactions between *S. thermophilus* and the thermophilic lactobacilli occur frequently. The lactobacilli can influence positively the early acidification by *S. thermophilus* during pressing: the pH reached at the end of pressing is the same, but the kinetic of acidification is modified, leading to differences in draining and subsequently to differences in calcium salts contents, texture, and quality of cheese opening (20). Interactions during pressing were recently more precisely evidenced in Comté cheese, using a factorial experiment plan (65). Regarding mechanisms, the main hypotheses are that *S. thermophilus* stimulates lactobacilli by CO_2, formate production and a quick release of galactose, and that lactobacilli stimulates *S. thermophilus* by a released growth factor, which could be a nitrogen source like peptides. Interestingly, mixed colonies of *S. thermophilus* and *L. helveticus* in Swiss cheese were observed frequently by scanning electron microscopy (Fig. 4) (45), which well supports the idea of a symbiosis between both species. By contrast, negative interactions can also occur because all these species are able to produce bacteriocins—for example, thermophilin 13 for *S. thermophilus*; lactacin A andB for *L. delbrueckii* subsp. *lactis* and lactocin 27; helveticin V-1829 and helveticin J for *L. helveticus*.

In natural starters involving growth multiplication in whey, such as in Italian cheesemaking, interactions between lactobacilli themselves, species or strains—either stimulations or inhibitions—were also observed (66,67), leading to complex population dynamics (68), but the mechanisms are not elucidated.

In conclusion, as long as mechanisms of interactions would no have been better characterized, the association of thermophilic LAB strains for cheesemaking will require a validation step by cheese assays, which is time-consuming and expensive.

B. Interactions Between Thermophilic LAB and Dairy Propionibacteria

Swiss-type cheeses like Emmental are characterized by an intensive development of dairy propionibacteria, mainly the species *P. freudenreichii*, during warm room ripening (69). Lactate is transformed into acetate, propionate, and CO_2 leading to the characteristic eye-

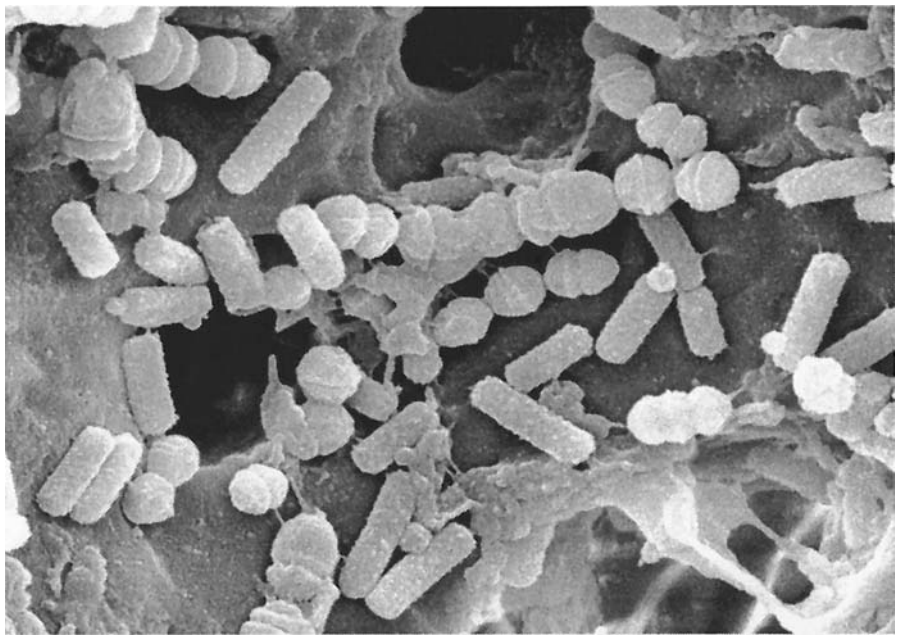


Figure 4 Mixed colony of *S. thermophilus* and *L. helveticus* in Emmental cheese, observed by scanning electron microscopy.

opening and nutty flavor of that cheese variety. The use of propionic starters in the manufacture of Swiss cheese has increased concomitantly with the decrease of the indigenous flora in raw milk. The kinetics and intensity of lactate fermentation by propionibacteria have a key role in the final quality of the cheese. Several investigations have shown that thermophilic lactobacilli were able to stimulate the growth of propionibacteria (21,70). The effect was shown to be largely strain dependent (71), the growth of some strains of *P. freudenreichii* being highly influenced by the chosen thermophilic lactobacilli, whereas other were not. The mechanisms were investigated by several authors (72–74) and the main hypotheses were (a) the nature of the lactate isomer produced (D or L) (b) the amount of amino acid and peptides released by the strong peptidases activity of thermophilic LAB. Recently, the positive effect of thermophilic lactobacilli on propionibacteria was attributed to their production of stimulating peptides, possibly peptides containing aspartate.

V. CONCLUSIONS AND PERSPECTIVES

From the data summarized here, several points can be underlined:

The use of thermophilic LAB starters (especially *S. thermophilus*) increases regularly, in particular in cheeses where they were not used before and which do not involve any cooking step.

Through genomic data, knowledge regarding their technological traits is increasing spectacularly (proteases, peptidases, metabolism, EPS production, transport system, phage sensibility).

The knowledge about technological traits of thermophilic LAB cannot be regarded as sufficient to achieve a better strain selection: mechanisms of interactions between them and with other cheese microorganisms such as propionibacteria, NSLAB, yeasts, and molds have to be further investigated (symbiosis, stimulation, complementarity in metabolic pathways, etc) because they are essential to the final sensorial quality of cheese.

ACKNOWLEDGMENTS

I am indebted to Jean-Louis Maubois, Jean-Francois Chamba, and Romain Richoux for fruitful discussions about thermophilic starters and for improving the manuscript.

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17

Manufacture of Cheese: Operational Procedures and Processing Equipment

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I. INTRODUCTION

Traditionally, cheeses have been produced in small households without any special equipment. By the end of the nineteenth century, cheese production (in Europe, New Zealand, Australia, and the United States) was conducted under more controlled conditions requiring special equipment, and proper cheese plants were built. With time the dairies have become larger and many types of equipment have been developed.

Most of cheese manufacture can be split into the same steps/stages; however, each step will depend on the cheese variety. The process flow in the production of hard and semihard cheese is illustrated in Fig. 1. In the following, each step will be clarified.

II. MILK TREATMENT

A. Removal of Undesirable Microorganisms

The fermentation of the milk itself will often inhibit the growth of many undesirable bacteria (spoilage or pathogenic bacteria), but in order to be sure and to avoid disadvantageous bacterial enzymatic activities, many producers prefer to inactivate or remove contaminating flora. This may prolong the shelf life of the cheese and protect consumers from illness. Inactivation is commonly performed by heat treatment (pasteurization) of the milk (e.g., 70–72°C [158–162°F] for 15–20 sec) using a plate heat exchanger. However, spores in particular may survive the heat treatment and cause severe problems during cheese ripening, particularly spores of *Clostridium tyrobutyricum*. The spores grow during cheese ripening and produce H_2 and CO_2, which may cause "blowing" of the cheese as well as butyric acid and other fermentation products with unpleasant smell. Methods such as bactocentrifugation or microfiltration can be used to overcome this problem. The bacto-

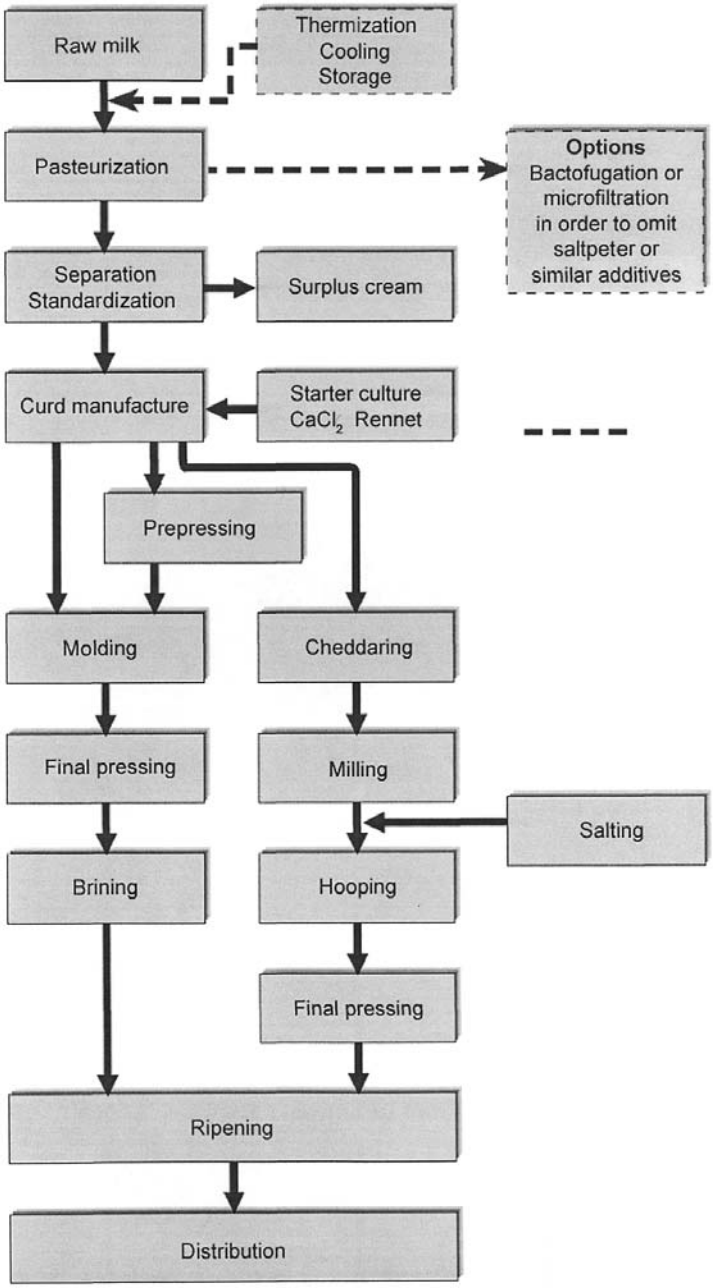

Figure 1 Process flow in production of hard and semihard cheese. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

centrifuge can separate bacteria and spores from the milk. Depending on the number of outlets on the apparatus top, they are classified into two types:

The two-phase bactocentrifuge has two outlets at the top: one for continuous discharge of bacteria concentrate and one for the bacteria-reduced phase.

The one-phase bactocentrifuge has only one outlet at the top: an outlet for the bacteria-reduced milk. (The bacteria concentrate is collected in the sludge space of the bowl and discharged at preset intervals.) (1)

Microfiltration is another method for separating bacteria and spores from the milk. In microfiltration a membrane with a pore size of approximately 1 micron is used to filter out bacteria from the milk. In order to avoid fouling the filter with milk fat, the milk is first separated into skim milk and cream, and only the skim milk is microfiltrated. The cream is heat-treated together with the retentate (e.g., at 118°C [244°F] for 4 sec). Afterward the cream is used for standardization of the milk (1).

B. Standardization

After the bacteria and spores have been removed, the milk is standardized by addition of cream after the separator by in-line mixing or by mixing with skim milk in tanks, followed by pasteurization. The composition of the milk varies over the year, influencing coagulation and syneresis. This can be overcome by the addition of calcium chloride (5–20 grams/100 kg); however, this is not always legal.

III. CURD MANUFACTURING

A. Addition of Starter Culture

Fermentation of the lactose to lactic acid gives cheese curds the desired pH. This fermentation is due mainly to addition of mesophilic lactic acid bacteria (e.g., *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc lactis* and *Leuconostoc mesenteroides* subsp. *cremoris*) or thermophilic bacteria (e.g. *Streptococcus thermophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) or by a combination of both. The activity of the starter cultures is very important in determining the different steps and their duration. The time used for milk ripening will be 30 min for most cheeses, during which only small amount of lactose is metabolized.

The starter cultures can be propagated locally as a mother culture and intermediate cultures before being added to the cheese milk or it can be added as a frozen or lyophilized concentrated culture directly to the cheese milk. Often the starter culture is added at the same time as the tanks are filled with milk in order to obtain uniform distribution and to quickly initiate the acidification as fast as possible. Acidification can be performed in open vats (Fig. 2); however, large modern tanks are closed and equipped with HEPA filters (in order to avoid contamination with bacteriophage), an automatically operated whey strainer, spray nozzles for proper distribution of rennet, and spray nozzles connected to cleaning systems (Fig. 3). All the tanks and tubes are designed so that they include an efficient cleaning-in-place (CIP) system, crucial to avoid bacteriophages. Today, tanks containing up to 22,000 liters are used.

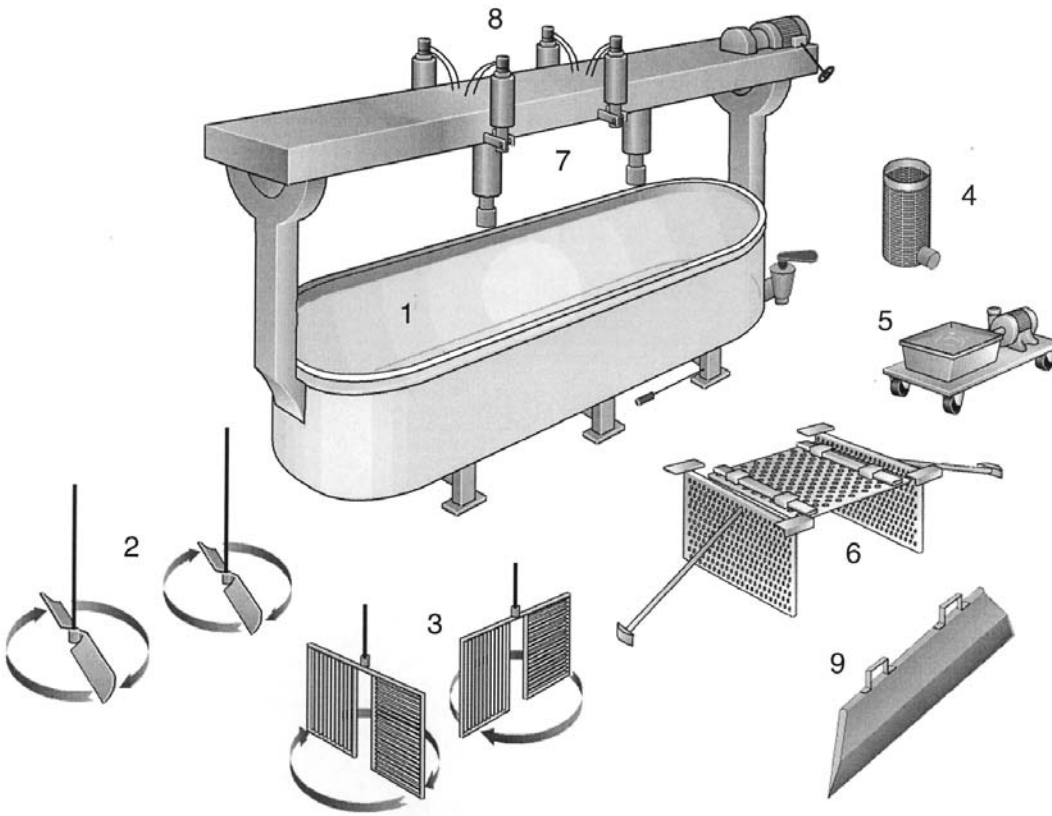

Figure 2 Conventional cheese vat with tools for cheese manufacture. 1, Jacketed cheese vat with beam and drive motor for tools; 2, stirring tool; 3, cutting tool; 4, strainer to be placed inside the vat at the outlet; 5, whey pump on a trolley with a shallow container; 6, prepressing plates for round-eyed cheese production; 7, support for tools; 8, hydraulic cylinders for prepressing equipment; 9, cheese knife. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

B. Addition of Rennet

Coagulation of milk casein is critical for proper curd formation, and it is achieved by the addition of rennet. The characteristic rennet enzymes are chymosine and bovine pepsin, which can be added either as purified standardized enzyme or as an extract of the fourth stomach of calves. The optimal temperature for rennet activity is approx 42°C (107°F). In cheesemaking, normal renneting temperature is around 30°C (86°F); renneting normally takes 20–30 min.

C. Cutting of the Curd

Renneting is followed by the cutting of the curd. This is a very important and effective procedure for whey release. When the coagulum has reached the required degree of firmness, it is carefully cut up and separated into small pieces (cheese grains) by tools equipped with knife blades or wires. By this separation, significantly better drainage of the whey inside the cheese grains is obtained (short distance to the surface). The cutting tools can be designed in different ways. Conventional open cheese vats are normally equipped with exchangeable pairs of tools for stirring and for cutting (Fig. 2). In a modern enclosed cheese-

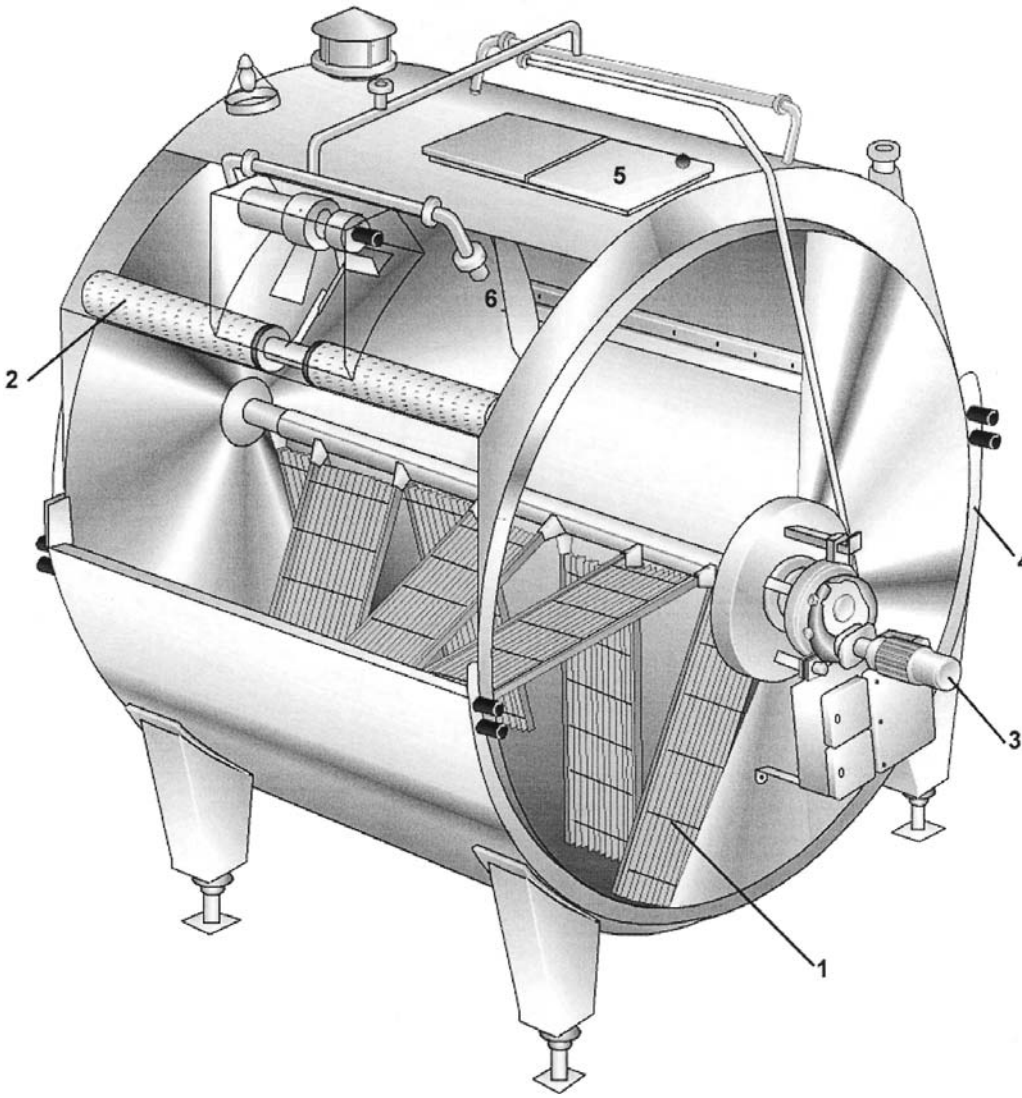

Figure 3 Horizontal enclosed cheese tank with combined stirring and cutting tools and hoisted whey drainage system. 1, Combined cutting and stirring tools; 2, strainer for whey drainage; 3, frequency-controlled motor drive; 4, jacket for heating; 5, manhole; 6, CIP nozzle. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

making tank, stirring and cutting are done with dual-purpose tools (cut or stir) depending on the direction of rotation (Fig. 3) (1). The cutting commonly takes about 10–15 min.

D. Stirring

After the cutting is finished, the curd is handled gently so that the cheese grains are not broken apart, which leads to loss of the fat in the whey. Stirring is done to assist in whey release (also named syneresis); the mechanical effect of stirring causes the grains to collide, and the pressure that results from the collision causes whey to be pressed out of the grains.

Obviously the effect is increased with a higher stirring speed and a decreasing distance between the grains (increasing drainage degree) (2). Stirring proceeds for one to two hours.

For some cheese varieties, a large portion of the whey is drained off before scalding is performed—for example, for semihard cheeses such as Gouda and Danbo, which requires about 15 min. Draining equipment is shown in Figs. 2 and 3.

E. Heating/Cooking/Scalding

The purpose of heat treatment is to regulate the pH and the moisture content of the final cheese. An increase of the cooking temperature will inhibit the growth of the starter culture, resulting in a higher cheese pH. Heating to temperatures above 40°C (104°F), is typically called cooking, and heating beyond 44°C (111°F) is called scalding). Also, the velocity of the heating influences whey release. Ordinarily, high velocity (short heating time) will result in a minor whey release and vice-versa. A high velocity increases the risk for blockage in the channel system of the cheese grains. The water-binding ability for the bound water in the curd is temperature dependent; water-binding ability decreases with increasing temperature, and vice-versa. Thus, high temperature will result in low moisture content of the cheese and vice versa. A too-low cooking temperature will cause the cheese to become soft and sour, while a too high temperature may result in cheeses that are dry and rubbery. For some cheeses, heating may only require adjusting the temperature to the original level; for other cheeses, a considerable temperature increase is necessary. The cooking of semihard cheeses made with mesophilic starter cultures is typically done at 34–40°C (93°–104°F) depending on the fat content, whereas scalding for hard cheeses made with thermophilic cultures is done at 50–56°C (122–133°F). In general, it can be said that it is best to heat gradually, approximately 1°C (1.8°F) in the first 5 min, 2°C (3.6°F) in the next 5 min, etc. The final temperature also has a significant influence on the later forming of the curd. The lower the temperature at which the cheese is formed, the more difficult it is to achieve proper pressing and to get the cheese grains to stick together (2). Depending on the type of cheese, heating can be performed in the following ways:

By introducing steam into only the vat/tank jacket
By introducing steam into the jacket in combination with the addition of hot water to the curd/whey mixture
By hot water addition to the curd/whey mixture only

Heating/ cooking is often ended by a final stirring that mechanically assists syneresis. The process, from the beginning of the heating to the start of the molding, takes about one hour for many types of cheeses, although, it can vary from 30–90 min.

During heating, the amount of lactose in the curd is regulated by the addition of water or the draining of the whey. Increased water addition decreases the concentration of lactose, resulting in a higher pH minimum. Addition of water also increases the moisture content. Therefore, water addition during the cooking of the cheese in the vat increases both the moisture content and the pH value of the cheese. If the water addition is exaggerated, however, a soft and foul-tasting cheese may result (3,4).

IV. MOLDING AND PRESSING OF THE CHEESE

A. Molding of the Cheese

After heating/cooking and stirring, the temperature gradually decreases, except for a few pasta filata–type cheeses. When the curd has reached its desired firmness and acidity, the

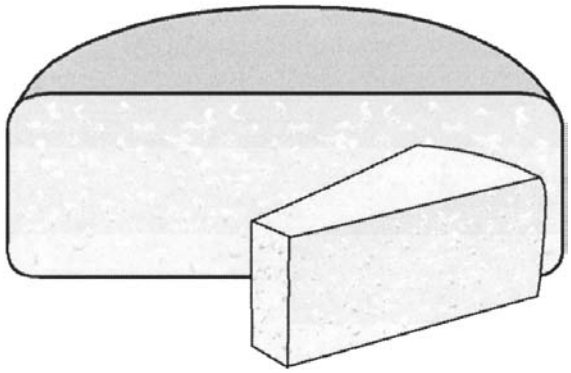

Figure 4 Cheese with granular texture. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

residual whey has to be removed and the cheese shaped. This is done via molding. Depending on how this is done, the final texture of cheese can be varied. If the curd grains are pressed under the whey, the grains will stick closely together and the texture will be very close and uniform. If the grains are separated from the whey before pressing, the grains will be intermixed with air, so that they cannot stick together and the resulting texture will be more open and granular. During ripening, the CO_2 produced from the citrate metabolism by *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc lactis*, and *Leuconostoc mesenteroides* subsp. *cremoris*, or produced from the lactate metabolism by *Propionibacterium*, will make the holes/eyes in the cheeses. Molding is in general made in the following ways (1):

1. The curd grains are transferred directly to molds, producing open-texture cheeses (soft and granular texture cheeses (see Fig. 4).
2. The curd grains are gently prepressed under the whey before molding, producing cheese with a closed texture (round-eyed cheese) (see Fig. 5).

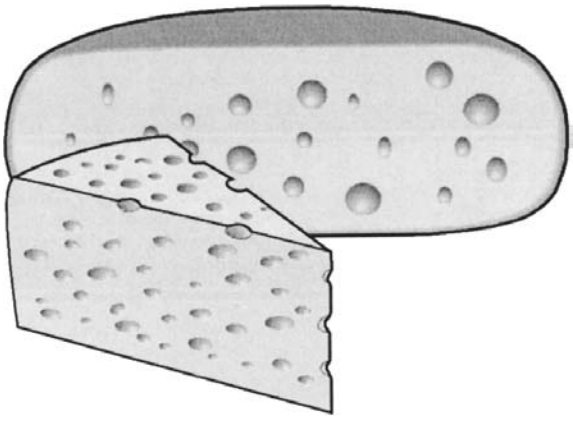

Figure 5 Cheese with round eyes. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

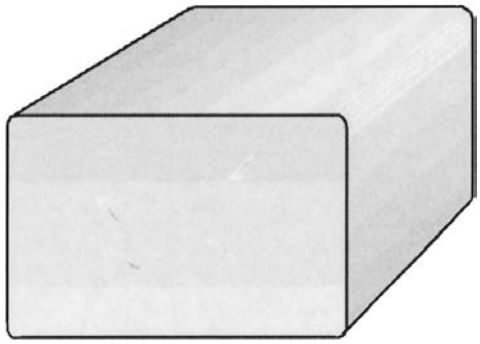

Figure 6 Closed-texture cheese with typical mechanical holes. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

3. The cheese curds are drained and salted before being piled and continually repiled to squash the curd, producing cheese with a closed texture, as shown for Cheddar in Fig. 6.
4. The cheese curds are drained before milling, warmed, kneaded and stretched before molding, producing a cheese with a compact texture (pasta filata cheese).

Procedures 1–4 in detail:

1. The whey and curd mixture is pumped across a vibrating or rotating strainer, where the grains are separated from the whey and discharged directly into molds (Fig. 7). Another method is to withdraw whey directly from manually operated open cheese vats.
2. The pressure during prepressing is applied gradually and should be at least 1–1.5 times the weight of the cheese curd at the end. The pressure is normally sustained

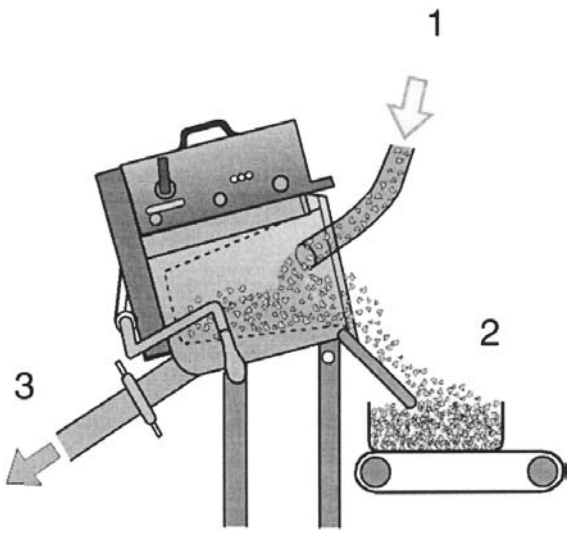

Figure 7 Curd and whey are separated in a rotating strainer. 1, Curd/whey mixture; 2, drained curd; 3, whey outlet. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

for 15–20 min. After being pressed, the curd is marked off into squares of exactly equal size and the pieces are cut out with a knife or with a special cutter as shown in Fig. 8.

3. Cheddar cheese is made by this procedure. It is very important that the acidification has reached the desired pH before molding because the cheese chips are dry salted before the molding. The salt will inhibit further growth of starter bacteria. Note that no holes (due to CO2 production) are wanted in Cheddar cheese.
4. The so-called 'Pasta filata' process is much like the Cheddar process. But when the cheese curd has finished acidification at pH 5.2–5.25, it is netted in warm water or in a salt brine at a temperature of 75–80°C (167–176°F), until the curd has reached a temperature of approximately 57°C (135°F). After this, the curd is formed in a mold and the formed cheeses are cooled.

Molding takes from 30 to 60 min. The acidification is continued during molding in procedure 1 and 2, but acidification must be complete before molding in procedure 3 and 4. A more detailed description of handling of different cheese curd types is available in Chapter 16. Figure 7 shows how curd and whey are separated in a rotating strainer, and Fig. 8 shows a mechanically operated prepressing vat with an unloading and cutting device. An advanced system, the Casomatic, is shown in Fig. 9. This machine can continuously do prepressing, block cutting, and molding (1).

B. Pressing of the Cheese

After having been molded or hooped (molds for Cheddar cheese are called hoops), the curd is subjected to a final pressing. This is done in order to press the last free moisture from the cheese and to form the cheese.

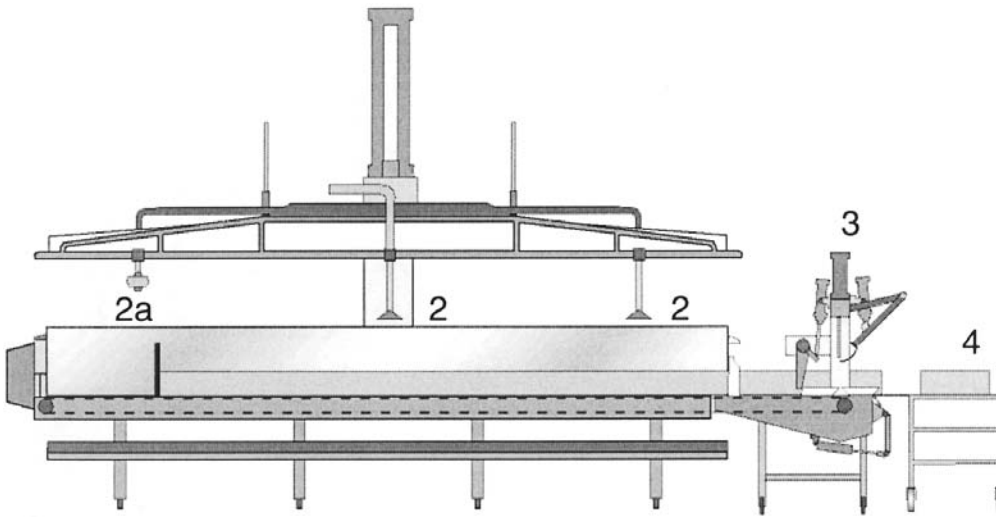

Figure 8 Mechanically operated prepressing vat with unloading and cutting device. 1, Prepressing vat (can also be used for complete pressing); 2, curd distributors, replaceable by CIP nozzles (2a); 3, unloading device, stationary or mobile; 4, conveyor. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

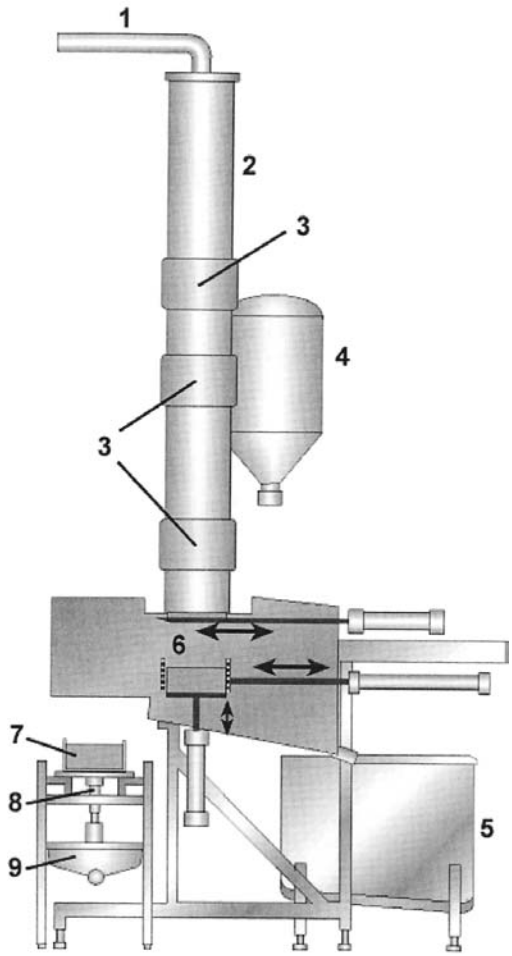

Figure 9 Casomatic, an intermittently operating continuous prepressing system, supplemented with mold filler. 1, Curd/whey mixture inlet; 2, column with sight glass (not shown); 3, perforated whey discharge; 4, interceptor; 5, whey balance tank; 6, cutting and cheese discharge system; 7, mold; 8, pawl conveyor; 9, whey collecting chute. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

The final pressing is initiated with a relatively low pressure because the rind may otherwise be too close before the whey is pressed out. This may result in pools of whey in the outer layers of the cheese and under the rind which can produce problems such as acid rind, spots in the outer edge, and fermentation under the rind. The pressure used in processing is different for the different types of cheese; it varies from 4 to 40 times the weight of the cheese, as shown in Table 1 (5).

To get uniform products, the pressing time is kept constant for each cheese variety, even if pressing time and pressure can substitute for each other to some extent. The proper time depends on several conditions, but normally round-eyed cheese is pressed longer than open-texture cheese, under equal conditions. Manually operated vertical and horizontal presses are available for small-scale cheese production as shown in Fig. 10. Pneumatic or hydraulic pressing systems simplify regulation of the required pressure. A more sophisticated solution is to equip the pressing system with a timer, signaling the operator to

Table 1 Examples of Pressure and Pressing Times

	Pressure		
Cheese	Kg/kg	Equal to ~ kPa	Time
Emmentaler	15–24	100–150	20 hr
Samsoe and Gouda	8	60	1–3 hr
Edam	6	50	1/2–1 hr
Danbo and Maribo	4–5	40	40 min
Esrom and Havarti	4–5	40	20 min

change pressure according to a predetermined programmer. For large-scale production, various systems are available. These include trolley table pressing, autofeed tunnel press, and conveyor press, as shown in Fig. 11 (1).

The pressing time is important for the moisture content in the cheese. Extension of pressing time and higher pressure give lower moisture content in the cheese. In addition to pressing time and pressure, the temperature during pressing is also a significant factor. Lowering the temperature during pressing gives cheeses with higher moisture content. Since cooling, on the other hand, is an effective means for rind fermentation, it will probably be acceptable to press at a relatively high temperature and then cool after pressing. Cooling can be done by putting the cheese in cold water after pressing (6).

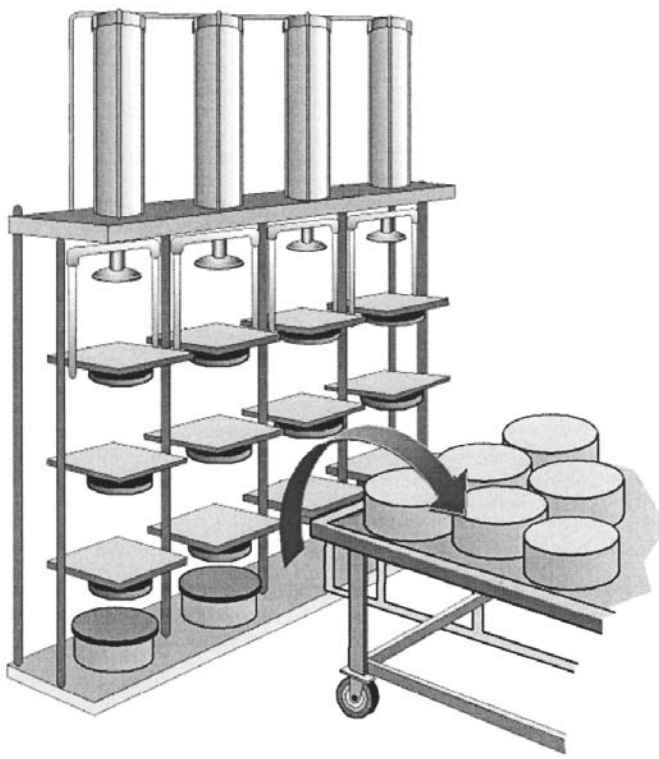

Figure 10 Vertical pressing unit with pneumatically operated pressing plates. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

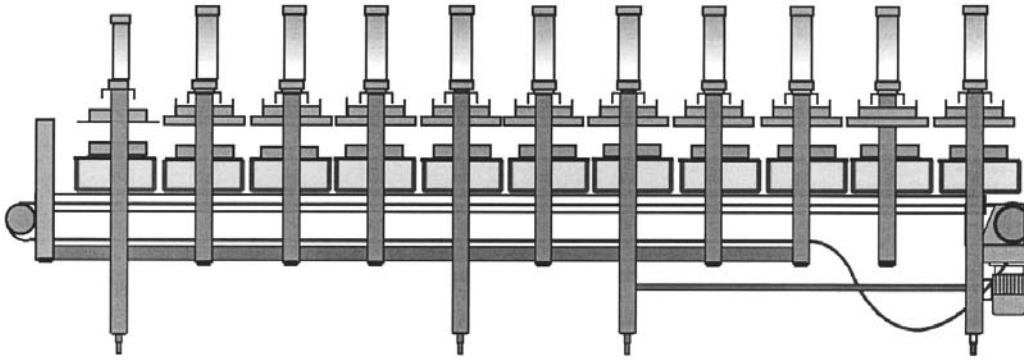

Figure 11 Conveyor press. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

V. SALTING OF THE CHEESE/BRINING

A. Salting Methods

The cheese curd is salted in order to balance the taste and consistency of the cheese and to preserve the cheese by inhibiting the growth of undesirable microflora. Salting of the cheese can be done by adding salt to the whey, by salting of curd after whey drainage, through dry salting by rubbing the surface of the cheese with salt (rind salting), or with brine salting. Often combinations of these methods are employed, but predominantly brine is used to salt the cheese. Brine salting systems of various designs are available, from fairly simple ones to technically very advanced ones. The most commonly used system is to simply place the cheese in a container with brine. A variety of systems based on shallow brining or containers for racks are available for large-scale production of brine-salted cheese. Different salting systems are shown in Figs. 12 and 13.

Brining is normally performed at temperatures no colder than 11°C (52°F), and if the fermentation in the cheese is slow, the temperature of the brine should rise to 22°C (72°F). The salt penetrates rapidly into the outer layer of the cheese and a high concentration of salt will stop fermentation of the remaining lactose in this layer. If the cheese is stored initially under dry and cold conditions, a leveling of this condition will take a long period of time and harmful fermentation can later occur under the rind. When there is a balance between whey draining, fermentation, temperature adjustment of the cheese, as well as swelling of the curd, brining can be done the day after the processing of the cheese. However, the specific technique for each kind of cheese and for each different processing plant varies and this must be taken into consideration.

B. The Salt Uptake of the Cheese

In the beginning, the cheese takes up salt rapidly, but for how long and to what extent it is absorbed depends on many conditions. The salting time is primarily dependent on the desired salt content in the cheese, but also depends on the following (5):

The temperature of the brine. Higher temperature results in more rapid salt diffusion.
Salt concentration in brine. More saturated brine gives quicker salt uptake but a more difficult condition for uniform distribution of salt inside the cheese.

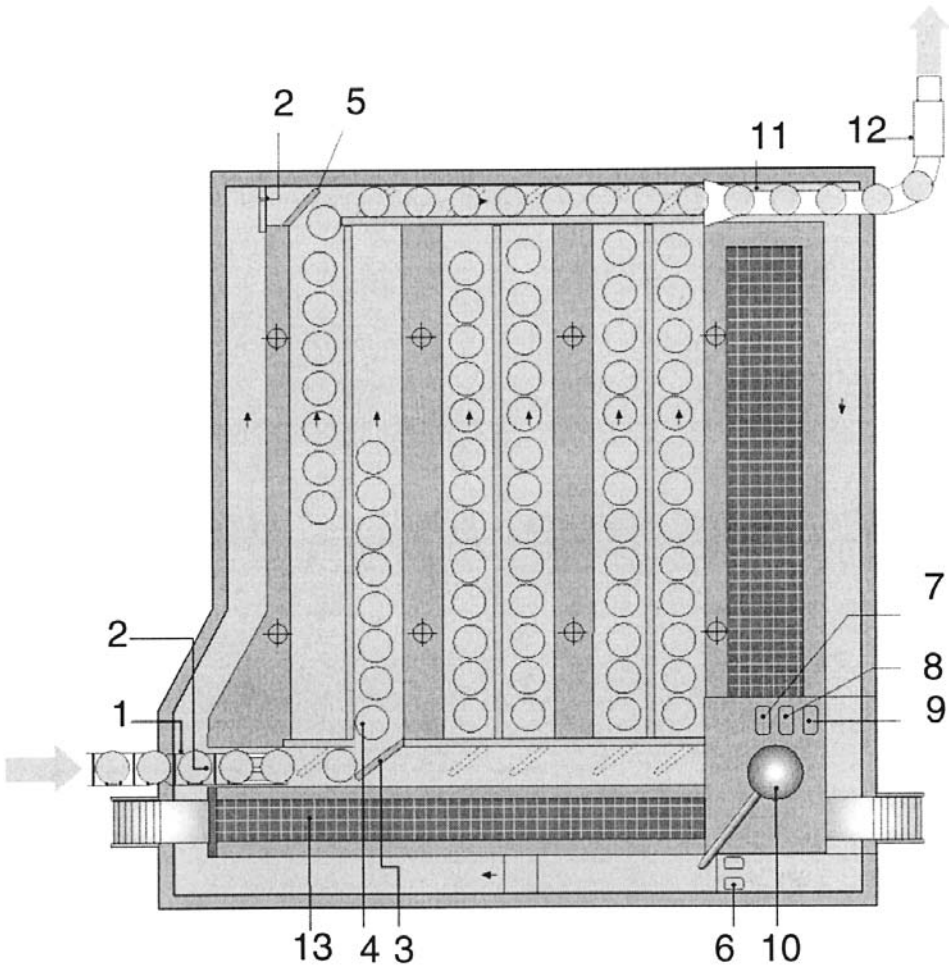

Figure 12 Surface brining system. 1, Inlet conveyor with sliding plate; 2, regulating screen; 3, inlet door with regulating screen and guiding door; 4, surface brining department; 5, outlet door; 6, twin agitator with sieve; 7, brine level control with pump; 8, pump; 9, plate heat exchanger; 10, automatic salt dosing unit (including salt concentration measurement); 11, discharge conveyor with gutter; 12, brine suction device; 13, service area. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

Shape and weight of the cheese. Flat cheeses take up salt very rapidly; square cheeses are salted slower than round ones.

Moisture in the cheese. Cheeses with high moisture content take up salt much more rapidly than quite dry cheeses. Hard cheeses, (e.g., Grana) will take up salt very slowly.

Acidity of the cheese. More acidic cheeses will take up salt more rapidly than less acidic cheeses.

Proper salting has a significant effect on the later development that takes place in the cheese. This includes effects on texture and flavor, and secondly effects on rind and color.

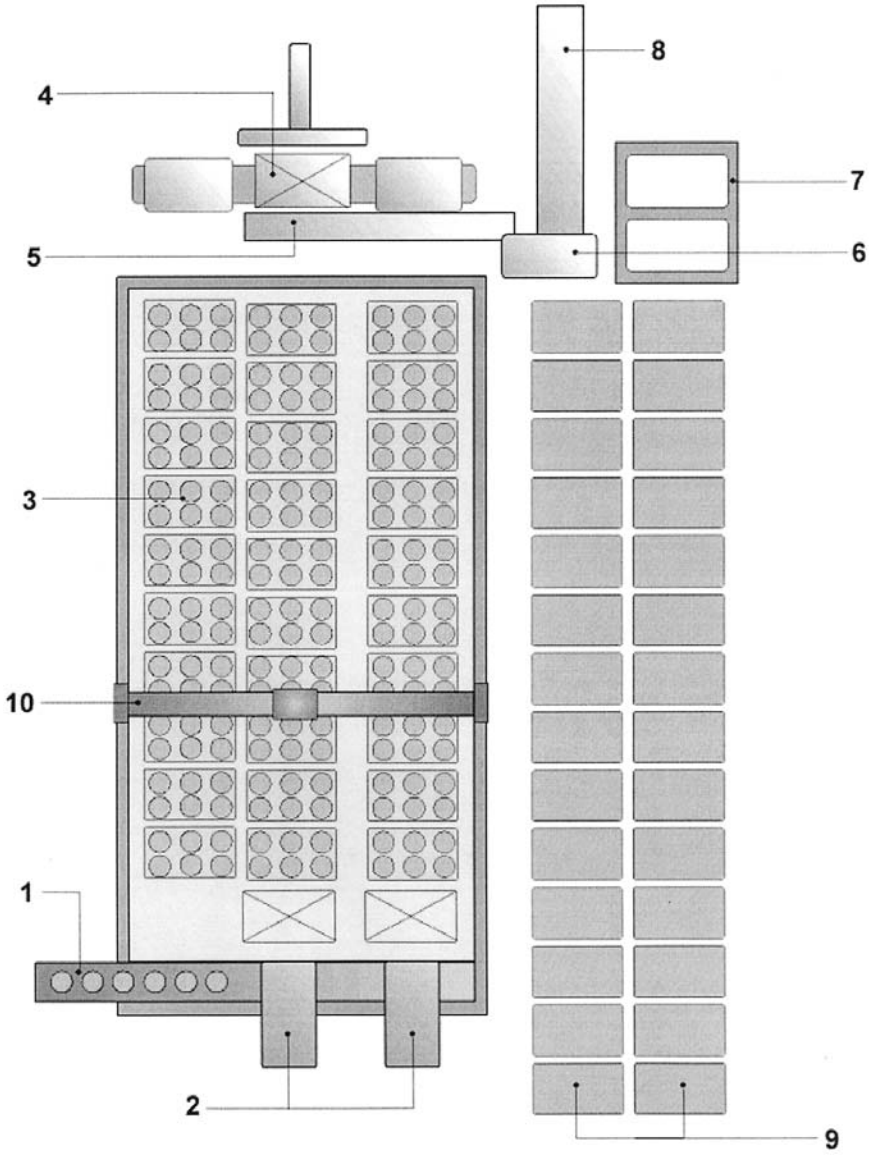

Figure 13 Rack brining system. 1, Feed conveyor; 2, mechanical loading station for brining racks; 3, brining racks; 4, mechanical unloading station for brining racks; 5, unloading conveyor; 6, lift; 7, rinsing bath; 8, belt conveyor; 9, space for empty racks and spare racks; empty racks can also be stored in the brine: If the cheeses are packed/treated immediately after brining, this area is not needed; 10, overhead traveling crane. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

Table 2 Salt Content of Different Types of Cheese

Cheese	% Salt
Cottage cheese	0.25–1.0
Emmentaler	0.4–1.2
Gouda	1.5–2.2
Cheddar	1.75–1.95
Limburger	2.5–3.5
Feta	3.5–7.0
Gorgonzola	3.5–5.5
Other blue cheeses	3.5–7.0

Source: Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.

A cheese salted too little will, in the beginning, have a flavor termed "flat," and a very elastic texture. Later the flavor easily becomes impure and the texture develops a chewy tendency. Too much salting results in a cheese with short texture and salty-bitter flavor. The initial rind formation will be affected by the brining, and the effect depends on the salt concentration of the brine.

Although a saturated salt solution (approx. 26% NaCl) can be used in salting, for open-texture and round-eyed cheeses a weaker concentration is often used, normally with 20–22% NaCl. The strength of the salting brine is determined by a hydrometer stating °Bé (Beaumé). At 15°C (70°F), 20°Bé corresponds to approx. 21% NaCl (21.2%) for a fresh brine. When the brine has been in use for a certain time, the hydrometer will show a deviation of 1–2° Bé because of substances dissolved in the brine. In practice, this means that, when measuring the strength of a 2–3 month old brine solution, degrees Baumé can be considered equal to the salt percentage. If the brine is much weaker than 20–22° Bé, the rind may become soft. If the brine is concentrated, there is the risk that the rind will become hard and low in moisture; brine that is too concentrated may cause cracks in the rind. The salting can be carried out in brining vats with circulating or nonmoving brine. If the brine is nonmoving, stirring must be done frequently.

The acidity of the brine should be about the same as that of the cheese (i.e., approx. pH 5.2), but in a freshly made solution it will usually be somewhat higher depending upon the acidity of the water supply; but the pH value should immediately be brought to the desired level by the addition of hydrochloric acid to the solution. The salt content in different types of cheese is shown in Table 2.

VI. STORAGE TREATMENT AND PREPARATION OF CHEESE

All cheeses except those consumed fresh must undergo a storage period for some amount of time. This is necessary in order for the enzymatic processes that determine the maturation of the cheese to have an appropriate reaction time. This reaction time varies significantly between different types of cheese, depending on which reaction takes place. For similar cheese types, it is common to use different storage times, depending on the

taste desired and the temperature used. The possibilities for varying the temperature are very limited; a change in temperature will change the ripening of the cheese.

A. Storage Treatment of Ordinary Hard, Semihard, and Soft Cheeses

The temperature that is chosen for the fermentation storage room depends on many factors, and the producer will choose an appropriate temperature in each case, according to the cheese type, milk quality, pasteurization temperature, acidification technique, moisture content, scalding temperature, etc. The commonly used fermentation storage room temperature for hard and semihard cheese is 17–18°C (63–64°F) and for soft cheese 15–17°C (59–63°F), but many producers use lower temperatures, and other use higher temperatures. The climatic conditions, temperature, and the relative humidity are of great importance to the rate of ripening, loss of weight, rind formation, and development of the surface flora (smear-treated cheeses—for example, Tilsiter, Havarti, and Esrom).

If a new production of surface-ripened cheese is started, it can be beneficial for development of a surface flora, to inoculate with a red smear culture, but in a continuous production, this is not always necessary. However, a smear former, which can be a starch porridge or premade smear former with *Brevibacterium linens*, is often used for surface-ripened cheese. The cheeses should be wiped often, especially in the beginning, in order to develop a smear layer that is healthy and not too thick. The wiping of the cheeses should also take place often in order to keep the growth of the blue mold fungi down.

Most cheeses are turned during storage in order to obtain an even evaporation and rind formation and to maintain a regular shape. The storage layout depends on the type of cheese. Installing permanent cheese racks in the store has been the conventional solution for both hard and semihard cheeses. Pallet racks or containers are a widely used system. Pallets or pallet containers can also be put on special wheeled pallets running on rails. This method also permits compact storage. Fig. 14 shows a ripening store based on pallets.

When the cheeses have reached an age of 2–3 weeks, they will usually be far enough along in their development that it is appropriate to lower the storage temperature. If conditions and space allow it, it is easiest to leave the cheeses at the same store and just lower the temperature and humidity. If the conditions and space are not available, the cheeses will have to be moved to a ripening store. The temperature and the humidity in the ripening store depend on how long it is desirable to keep the cheeses in storage. The temperature, humidity, and ventilation of the ripening store depend on how long the cheese will be stored there. If the cheeses are only to be stored 1–2 weeks because they are to be sold as 6-week-old cheeses, it is possible to have a relatively dry ripening store with approximately 85% humidity and a temperature not above 12°C (54°F). On the other hand, if the cheeses are to be left in the ripening store for a longer period, it is possible to lower the temperature and the humidity in consideration of the shrinkage and rind formation of the cheese.

Wiping of surface-ripened cheeses in the ripening store is not necessary if they are removed within 1–2 weeks. But it may be necessary to apply water or smear former to the surface of the cheese about once every 14 days if they are to be stored in the ripening store for a longer period.

When the cheeses are matured and have fermented enough to be stored without risking post-fermentation, the cheeses are cleaned, marked, and coated with paraffin plastic or wax in the usual manner, and are then transported to the cold store right after paraffining, plastic-coating, or wax coating. The air temperature of the cold store should be kept at 2–4°C (36–39°F), and the air should be dry.

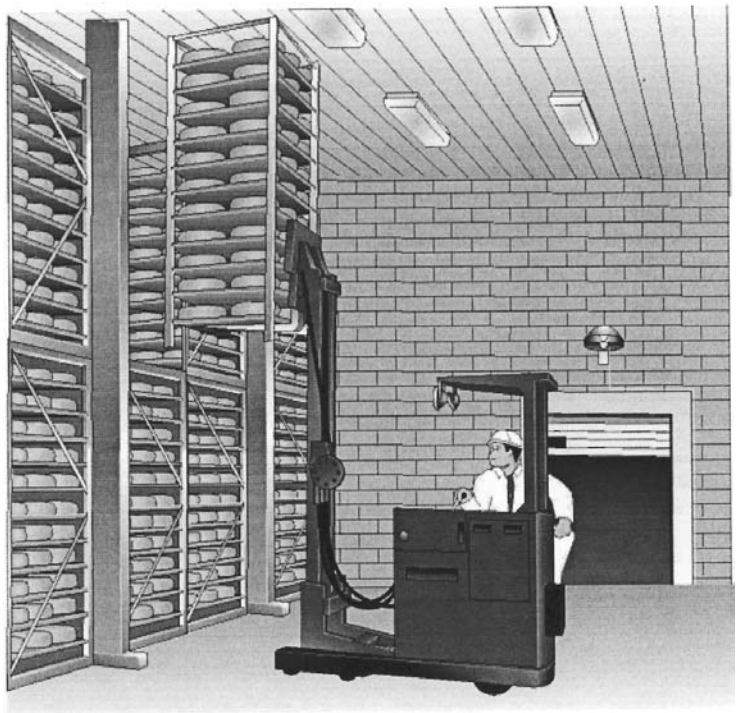

Figure 14 Cheese storage using pallets. (From Ref. 1, courtesy of Tetra Pak Processing Systems AB, Sweden.)

B. Storage Treatment of Rindless Cheeses

The rindless cheeses are placed in special storage boxes consisting of a bottom and a lid, or in plastic-boxes or containers. This is necessary to preserve the cheese's form and to facilitate stacking of the cheese during storage. Shelves are then not needed in the storage store, and as a result a significantly higher storage capacity is obtained. Stacking is also important to achieve a constant pressure on the foil of the cheese; pressure has the effect of preventing pocket-formation between the cheese and the foil. Of course, cheeses at the top are under less pressure and therefore, must be moved around regularly to ensure that the cheeses on top are rotated at some point to below. It is also good to turn some cheeses. How often the cheeses should be turned depends somewhat on the cheese's type, but once a week for the first 2 weeks would be safe. Later in the process the frequency can gradually be decreased. Different types of cheese require different temperatures and relative humidity in the storage rooms—ordinary hard and semihard cheeses for example, with 10–12°C (50–54°F) and a relative humidity of 60–70% (7,8).

C. Storage Treatment of Mold Cheeses

Mold cheese such as Blue Cheese, Danablu, Camembert, and Brie are kept in so-called dripping rooms after the cheese mass has been molded, at a temperature of 20–25°C (68–77°F) for approximately 12 hr (Blue Cheese and Danablu, 36 hr). After salting and drying, the cheese is transferred to a ripening store, which is important because its temperature

and humidity correspond to that of a mountain cave. Blue Cheese and Danablu, however, are "pierced" first, before they are transferred for ripening at a temperature of approximately 10°C (52°F) and a humidity of 95–98%. Camembert and Brie are kept for ripening at a temperature of 13–15°C (55–59°F) and a humidity of 85–90%. It is important that mold cheese be stored with a very low circulation of air, for instance by "bag-cooling." After a ripening period of approx. 5 weeks, the Blue Cheese and Danablu cheeses are washed. Camembert and Brie are ripe after 2–3 weeks. The cheeses are then ready for packing and sale.

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18
Packaging of Cheeses

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I. INTRODUCTION

Packaging of cheeses has evolved from being used for protection and transportation of the product from the cheese shop or local dairy to homes into being a multifaceted task involving not only chemical, physical, microbiological, and sensory aspects but also issues such as marketing and sales, legislation, environmentally sound production schemes, and consumer-related parameters (e.g., openability and overall convenience). Convenience aspects and long distribution times have resulted in increased use of modified atmosphere packaged products, the application of active packaging concepts, and market introduction of products that are sliced, grated, cubed, or otherwise manufactured to provide easy-to-use consumer products. The introduction of new packaging concepts has to some extent been substantiated by research. However, the somewhat empirical pack-and-pray approach still dominates commercial usage of many aspects of cheese packaging technology.

This chapter briefly describes the major quality deteriorative processes in cheese and how these may actually be prevented or minimized by packaging. An overview of present cheese packaging concepts is included, and finally, future areas of research and development are pinpointed.

II. EFFECT OF PACKAGING ON QUALITY CHANGES IN CHEESES

Major quality changes in cheeses affected by packaging include:

Physicochemical changes due to oxidative reactions
Physical changes such as water loss and subsequent rheological alterations
Microbial changes, including survival and growth of both desirable and undesirable microbes

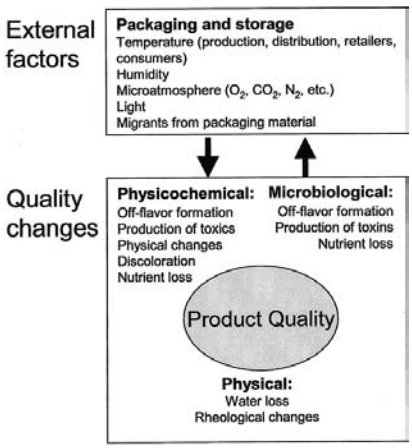

Figure 1 Interplay between external factors within the food packaging sphere and quality changes taking place in the product.

The above-mentioned changes all affect sensory aspects and thus influence how consumers perceive the products.

The interplay between internal and external factors within the food packaging sphere and quality changes occurring in the product is outlined in Fig. 1.

A. Physicochemical Quality Changes

The major physicochemical quality-determining factor in cheese is oxidation, induced either thermally, enzymatically, by the presence of transition metals, or by light exposure. Irrespective of the induction mechanisms, these changes result in off-flavor formation as the primary reaction products from lipid oxidation, the lipid hydroperoxides, are decomposed into volatile secondary oxidation products such as alkanes, alkenes, aldehydes, alcohols, ketones, esters, and acids (1,2). Furthermore, oxidative reactions involving proteins, vitamins, and pigments occur, which result in discoloration, nutritional loss, formation of carcinogenic compounds, and changes in physical characteristics (3,4).

Light-induced oxidation plays a key role in relation to packaged cheeses. Cheeses may be exposed to light from natural and/or artificial sources during processing, storage, distribution, and marketing, resulting in oxidation of lipids, proteins, and sterols. Additionally, many vitamins (e.g., ascorbic acid, riboflavin, vitamin A, β-carotene, and tocopherols) may be destroyed when cheeses are exposed to light. Some of these are degraded by the direct effect of light; others indirectly, by reaction with active oxygen species formed during light-induced oxidation.

Photooxidation may take place by *photolytic autoxidation* or *photosensitized oxidation*. Photolytic autoxidation is a direct formation of free radicals initiated by high-energy light such as sunlight (10,000–100,000 1x) (5). However, visible light may also trigger oxidative changes due to the presence of photosensitizers (e.g., riboflavin in cheeses) and oxygen (photosensitized oxidation) (6). Riboflavin is an efficient photosensitizer: it readily absorbs visible light energy, thereby exciting the sensitizer to a higher energy level, which makes for reactions with (for example) unsaturated free fatty acids or produces the very

reactive singlet oxygen ($^1O_2$). Subsequently, the $^1O_2$ reacts with the unsaturated free fatty acids, producing free radicals, lipid hydroperoxides, and finally volatile carbonyl compounds. Many of these photochemical reactions result in autocatalytical oxidative processes, implying that even short light exposure time may have detrimental effects on the stored product and that oxidation, initiated by light, may continue even when the cheeses are protected from light.

Cheeses are exposed to different light sources during processing and distribution and at the retailers. Light with high quantum energy (i.e., lower-wavelength light in the visible/UV spectrum) has the potential for the most severe quality deteriorative effects (4,7) because this light can be absorbed by a variety of molecules. Packaging materials absorb most of the energy-rich UV light, and thus UV light is generally not so harmful to the packaged dairy product as is light in the blue-violet region (400–500 nm) of the spectrum. Light in this wavelength range is absorbed by the two major colorants of milk—the prooxidant, riboflavin, and the antioxidant, β-carotene—and is thus critical with respect to photooxidation. The balance between the concentrations of these two compounds, which is largely determined by feeding (8) and dairy unit operations, determines to which degree light in this wavelength range impairs product quality during retail storage. A recent study on the effect of specific wavelengths present in commercial fluorescent light (366 nm, 405 nm, 436 nm, and storage in the dark) revealed that visible light was more detrimental than was near-UV light and dark storage (9). Furthermore, the study indicated that 405 nm exposure was more detrimental to semihard Havarti cheese than was 436 nm. Further studies evaluating the effects of specific wavelengths are called for.

Increasing light intensity (i.e., the photon flux) affects light-induced oxidation negatively (10,11). Intensities found in the literature vary from approximately 40 to 12,000 1x in dairy displays, with average intensities of 1000–3000 1x (5,12,13). Intensity results from different studies can most frequently be compared on a qualitative basis only because the light sources seldom have identical spectral distribution of radiation.

Limited temperature dependence is expected for photochemical processes because these reactions have low activation energies (14). Very recent studies substantiated the limited temperature dependence for photooxidation of semihard cheeses at 3°C and 10°C (15). However, autoxidation is temperature dependent and may prevail when the initial free radicals are formed through photooxidative processes. Photooxidative changes increase with storage time progression (9,15–20).

Important packaging parameters for prevention of photooxidation encompass the following:

1. Initial Gas Composition

Oxygen is required for oxidation to occur, and hence, minimizing oxygen levels is effective in preventing oxidation. Factors influencing the amount of O_2 include initial gas composition (both dissolved in product and present in headspace), product-to-headspace volume ratio, product respiration (i.e., microbial and enzymatic conversion of O_2 to CO_2), and oxygen transmission rate of the packaging material (4).

2. Surface Area and Product-to-Headspace Volume Ratio

Photooxidation takes place mainly on the surface of the cheese (21). Thus, increasing the surface area leads to more severe photooxidative effects (10,15). Factors such as mobility and diffusion of oxygen into the product have not yet been researched; consequently, such evaluations are essential.

3. Packaging Materials

Light protection offered by the packaging material depends on numerous factors, which may be altered in order to provide the necessary protection against photooxidation of specific dairy products:

Type of material (oxygen transmission, light transmission) (7,20)
Wall thickness/grammage (7,22,23)
Processing (including orientation of the polymer, crystallinity, and incorporation of additives) (7)
Inks/pigmentation/cavitation (20)
Metallization (7)
Attachment of labels (23)

Recent reviews on photooxidation in dairy products include Bosset et al. (24), Skibsted (3), Thron (25), and Mortensen et al. (4).

B. Physical Quality Changes

Packaging may affect physical changes such as water loss at the surface (26,27) resulting in color changes (a more intense color) and rheological alterations in the cheeses (28–30).

Important packaging parameters affecting water loss and the texture include the following:

1. Composition, Volume, and Humidity of the Initial Gas Atmosphere

The gas composition may affect water loss. Gonzales-Fandos et al. (31) found that the water loss in fresh goat cheese (Cameros cheese) packed in 100% CO_2 was 10.7% compared to a water loss of approximately 3% for Cameros cheese packed in either 20% CO_2/80% N_2, or 40% CO_2/60% N_2. The differences may be attributable to reduced pH in the cheese packed in 100% CO_2 and/or a lower water content of the CO_2 gas compared to N_2. Furthermore, a gas composition consisting of 100% CO_2 may affect the texture negatively, probably due to increased water loss (32).

In addition, the headspace volume and humidity of the gases are presumed to affect the rate of water loss. However, literature in this area is nonexistent.

2. Packaging Material

Water vapor permeability is essential for water loss. The water vapor permeability depends on numerous factors, including humidity, the type of material, and the wall thickness/grammage. Desobry and Hardy (33) noted that a 2.5–5% weight loss of cheese due to insufficient barrier properties is normal. They also found that dehydration of fresh cheeses should be avoided, because a dehydrated surface is a major quality defect in these products. Topal (34) noted a 8.5% weight loss of Kashar cheese during traditional curing, which could be prevented by maturing the product in Cryovac films, thereby increasing profitability. British Standard (35) recommended water vapor permeabilities of less than 30 g m^{-2} day^{-1} for consumer packages and 4.0 g m^{-2} day^{-1} for gas and vacuum packaged cheeses (25°C, 75% RH). Obviously, the required permeability may vary from product to product. Weight loss of unpackaged versus packaged Camembert cheeses was also reported to be different (36).

C. Microbial Quality Changes

Even though cheese was originally developed as an efficient way to store the nutritional part of fresh milk for an extensive period, the product is still susceptible to microbial degradation. The types of organisms capable of surviving in cheese are, however, quite different from the ones present in milk, due to the low pH (4.5–5.2) and the moderate to high salt content. The specific group of organisms growing on a cheese is termed the associated microbiota of the cheese. The composition of this microbiota is strongly affected by the chemical and structural composition of the cheese, which again depends on type of milk, production, maturation, and storage conditions. In order to avoid microbial changes through proper packaging, it is of great importance to know how the cheese is produced, its microbiota, and to have some general knowledge about its chemical composition. It is an established fact that the primary spoilage organisms in cheese are molds. Furthermore, yeasts may cause problems in products such as Feta and decorated cream cheese (i.e. cream cheese covered with herbs, nuts, spices, etc.); in fact, lactic acid bacteria may spoil decorated cream cheeses.

1. The Origin of Microbial Problems

All cheese production starts with mechanical pretreatment of the milk, from simple filtration to remove larger particles (e.g., dirt), to adjustment of fat content, to homogenization or ultrafiltration. These processes have only limited direct effect on the microbiological status of the milk unless the milk is sterile-filtrated. The subsequent heat treatment is important in controlling the number of microorganisms transferred from the raw milk to the cheese. The microbiota associated with the raw milk includes nonstarter lactic acid bacteria, yeasts, and molds. Nonstarter lactic acid bacteria and some yeasts exhibit beneficial impact on maturation and the sensory quality of cheese (37,38), whereas other yeasts and bacteria may lead to spoilage.

Hygiene in the dairy plant is also an important issue because most microbial problems arise from insufficient sanitation. This is, however, a very complex issue. Several authors address the cleaning and disinfection regimens applied in dairy plants in order to minimize the load of bacteria, yeasts, and molds (39). Others have tried to track down and map distribution of spoilage organisms within the dairy plants and how these organisms actually entered the plant facilities (40,41).

Finally, decoration and coating of cheese pose a great risk of contamination because the spices, herbs, or nuts used may contain yeasts and molds (42).

2. Cheese as a Substrate for Spoilage

The following includes an overview of different microbial changes likely to occur in cheese.

Listeria is one of the most feared microorganisms within the dairy industry. Most dairies have a zero tolerance for *Listeria*, and based on the apparent growth domain of *Listeria*, this would make sense, because some strains of *Listeria* may grow at pH levels as low as 4.1, at a temperature of 0.5°C, at water activity levels below 0.93, and in environments containing 10% NaCl. Additionally, the species thrive on anaerobic conditions. Fortunately, growth at these extreme conditions is only possible when otherwise optimal conditions exist. Thus, *Listeria* has primarily been a problem in soft, low-salt, mold-ripened cheeses produced under improper pasteurization or inadequate hygienic conditions. These cheeses are more susceptible to *Listeria* because the fungal metabolism during ripening will make the pH rise considerably in the center of the cheese. Based on results

from experiments on fermented meat products, it appears that modified atmosphere packaging with high CO_2 content would serve as an effective protection (43,44).

Late blowing is another economically important spoilage process of cheese. It is attributable to the growth of *Clostridium tyrobutyricum*, *C. butyricum*, and related species, which produce CO_2 by butyric acid fermentation (45). These are mesophiles, so they will not cause spoilage in chill-stored cheeses.

Nonstarter lactic acid bacteria may in some cases cause problems in modified atmosphere packaged, decorated cream cheese (42), because the bacteria are facultative anaerobic, which implies that they cannot be controlled by modified atmosphere packaging.

Yeasts constitute an important group of spoilage organisms, especially relative to products with moderate to low salt levels such as cream cheese and cottage cheese or products stored in brine, (e.g., Feta). Yeasts are nonpathogenic and do not produce mycotoxins. However, several species produce undesirable off-flavors and may cause changes in color and texture. Deformation of the package may take place due to gas production by fermentative yeasts such as *Torulaspora delbrueckii*, which causes swelling of Feta cheese (42). A range of yeasts spoils Feta, and several of these are restricted to a few dairies, where they dominate. The most important spoilage yeasts of Feta are *Debaromyces hansenii*, *Klyveromyces maxianum*, *Yarrowia lipolytica*, and *Candida butyri*. Other important yeasts include *T. delbrueckii*, *K. lactis*, *C. sake*, and *C. butyric* (42). In cream cheese, the most important spoilage yeasts include *T. delbrueckii*, *C. parapsilosis*, *Pichia fermentans*, *D. hansenii*, and *Y. lipolytica*. Total removal of oxygen by applying an oxygen scavenger does not fully preclude yeast growth. Combinations of very low oxygen levels and high carbon dioxide levels may inhibit growth of the weakly fermenting yeasts (e.g., *D. hansenii*), whereas the strongly fermenting species, *K. lactis* and *T. delbrueckii*, are nearly unaffected (46).

Molds are the dominating spoilage organisms of cheese. Several fungi have been isolated from cheese but only a small number are actually significant spoilers (47). Most important is *Penicillium commune*. Two species, *P. nalgiovense* and *P. verrucosum*, dominate in cheese with high salt content, and *P. solium* and *P. nalgiovense* appear mainly in cheese stored at temperatures below 5°C. *P. roqueforti*, which is used in the manufacturing of blue mold cheese, also appears to be a contaminant in inadequately vacuum packaged cheeses. Haasum and Nielsen (48) did a detailed study of the physiology of cheese-associated fungi. One of the main conclusions of this study was that fungi resistance to reduced water activity (or high salt) was in fact linked to sensitivity to high carbon dioxide levels, corresponding to the normal conditions on the surface of the cheese. Other species such as *P. roqueforti* and *Geotrichum candidum* are less affected by high carbon dioxide levels; however, they are strongly affected by reduced water activity, which corresponds with their affinity for the center of the cheese. This is important relative to optimizing the packaging conditions for cheese, because it indicates that the normal surface fungi (*P. commune*, *P. camemberti*, *P. solitum*, *P. nalgiovense*, and *P. caseifulvum*) are effectively controlled by high carbon dioxide levels.

Studies of growth at very low oxygen levels showed that most fungi were inhibited only when the O_2 levels were less than 0.5%, and levels as low as 0.01% were required to efficiently inhibit any and all fungi (71). Most resistant to very low oxygen levels were the Fusarium species and yeasts, which all showed restricted growth (<2 mm) after 1 to 2 weeks at 25°C with oxygen absorbers ($O_2 < 0.01\%$). On an optimal growth medium, all cultures reached almost the same size irrespective of the initial amount of O_2, whereas the combination with high CO_2 levels retained growth. Another study indicated that packaging gas had a pronouced effect on survival of spores on the cheese (49). Storage at high

carbon dioxide levels effectively prevented growth and preserved the conidia spores effectively, whereas packaging at lower carbon dioxide levels allowed the conidia to germinate but not grow, which eventually led to their inactivation, resulting in a longer shelf life for the product.

Modified atmosphere packaging is in some cases combined with preservatives to ensure product stability and safety even after opening. Sorbates are mainly used for cream cheeses and processed cheeses, whereas hard cheeses may be immersed in a solution of natamycin (pimaricin, E235) or covered by a plastic coating containing natamycin and sorbate. Used alone, these preservatives cannot completely hinder spoilage because a few fungal species are resistant to either sorbate or natamycin (50,51). Here, as in all other cases, optimal product safety is obtained by combining several product-specific preservation factors.

III. PACKAGING OF DIFFERENT CHEESES

Optimal packaging solutions may prevent or minimize quality changes, thus resulting in both increased shelf life and maintained quality. Packaging requirements are linked to the practices and goals of the producer and include initial product composition, production line requirements, marketing aspects, secondary packaging method (including packaging used for display of the products), distribution method, shelf life required, waste treatment practices, and price. Hence, different types of cheeses call for different types of packaging concept. The following lists examples of packaging materials used for packaging of individual products, and as such can be used for inspiration. Table 1 briefly summarizes important characteristics of packaging materials used for cheeses, both as monolayers and as part of more complex packaging concepts. Final selection should be based on extensive evaluations and should be carried out in collaboration with the packaging suppliers.

Cheeses are normally classified according to their moisture level (53). However, the following subdivision of cheeses focuses on their packaging requirements rather than on their composition.

A. Fresh Cheeses

This category comprises slow-drainage, acidified cheeses characterized by a moisture content greater than 80% (54,55). Main types of fresh cheese include cottage cheese, quark, cream cheese, and Petit Suisse. The packaging should protect these products against light, oxygen, loss of moisture and aroma, and contamination, as well as provide sufficient mechanical stability.

Most fresh cheeses are packaged in atmospheric air due to the short shelf life required. However, modified atmosphere packaging may be used to prevent mold growth (e.g., in cottage cheese or in decorated cream cheeses). Gases with low residual oxygen and high carbon dioxide levels may be used to ensure microbial stability (56–59).

Cream cheeses are pasteurized prior to packaging, and therefore spoiling microorganisms may stem from cross-contaminations (surroundings or packaging materials). Hot filling of the product may be applied to overcome cross contamination from the packaging materials. Some of the cream cheeses are decorated with spices, herbs, nuts, or fruits, which also may be a source of contamination. These cheeses are typically packaged in atmospheres with high CO_2 contents. Gonzalez-Fandos et al. (31) noted that for

Table 1 Characteristics of Materials Used for Cheese Packaging

	Structure	Mechanical properties	Moisture permeability[a]	Oxygen permeability[a]	Applications (examples)
Low-density polyethylene, PE	Ethylene units; density 0.917–0.924 g/cm^3	Tough, extensible, flexible, sealability	Medium/low	High	Foils
Polypropylene, PP	Propylene units	Moderate stiffness, strong Withstands higher temperature than PE	Low	High	Extruded or injection molded cups/beakers, foils
Ethylvinyl acetate, EVA	Copolymerization of low-density polyethylene and 1–20% vinyl-acetate	Tough, highly extensible	Medium	High	Foils
Polystyrene, PS	Styrene units	Stiff, strong, brittle	High	High	Cups, foils
Polyethylene terephthalate, PET	Ethylenglycol and dimethylterephthalate or terephthalate-acid	Stiff, strong High temperature resistance when biaxially oriented	Medium	Low/medium	Foils, thermo-formed cups
Polyamide (Nylon), PA	Nylon 6: Polymerization of caprolactam	Stiff, strong Temperature resistance	Medium	Low when dry	Foils
Polyvinylidene chloride, PVdC (Saran)	Vinyliden units	Tough, soft Withstands hot-filling High heat sealability	Very low/low	Very low	Lacquers/coatings (barrier layer) Foils
Ethylvinyl alcohol, EVOH	Ethylvinyl-acetate and methanol	Strong, stiff Thermally stable	Medium/high	Very low/ extremely low below 75% RH	Barrier layer
Cellophane	Chemically modified cellulose	Stiffness, no heat sealability	Medium/low	Low (coated)	Wrapping
Paper/paperboard/ parchment	Cellulose, hemicellulose, lignin, etc. Parchment: Sulfuric acid treated	Stiffness Strong, isolating Parchment: High wet-strength; oil and grease resistant	High	High	Secondary packaging Multilayer packaging
Glass	SiO_2, NaCl, $CaCO_3$, returnable glass	Strong, stiffness Grease resistance	Very low	Extremely low	Jars, coatings (barrier layer)
Aluminum	Aluminum oxides and cryolite	Grease resistance Lightweight	Very low	Extremely low	Foils, lids, cups, tubes

[a] Subdivisions regarding moisture and oxygen permeability are based on guidelines in Ref. (52). Moisture permeability ($gm^{2}\text{-}d^{-1}$, 38°C, 90% RH): Very low: <1; low: 1–10; medium: 10–50; high: >50. Oxygen permeability ($mLm^{-2}d^{-1}atm^{-1}$, 20°C, 0% RH): Extremely low: <1; very low: 1–10; low: 10–100; medium: 100–1000; high: >1000.

Cameros goat cheeses, 40–50% CO_2 levels (N_2 as filler), increased shelf life while maintaining sensory acceptability. 100% CO_2 was optimal; from a microbiological point of view, however, the high CO_2 levels impaired the sensory quality.

Selection of packaging materials as well as the use of modified atmosphere packaging should be based on the shelf life required. Examples of packaging materials currently used include polystyrene (PS), polypropylene (PP), glass jars, nitrocellulose-coated cellophane, and polyethylene (PE)/polyamide (PA) combinations to obtain oxygen barriers in modified atmosphere packaged products. Lids made of printed aluminum foil with heat-sealable lacquer are often used. Coated aluminum lids are used for products with high salt content, such as Feta cheese, in order to avoid corrosion.

B. Semihard and Hard Cheeses

Matured cheeses are ripened by bacteria. The category includes cheeses such as Edam, Gouda, Danbo, Havarti, Cheddar, Gloucester, Derby, Leicester, Gruyère, Emmental, Mozzarella, Provolone, and very hard cheeses such as Parmesan and Romano (Italian-type cheeses).

These cheeses require an adequate water vapor barrier as well as low oxygen levels, obtained through the use of high oxygen barrier materials and/or gas packaging. Furthermore, the amount of carbon dioxide should be controlled to avoid unwanted blowing or collapse of the package. Packaging methods include flow packaging, hard and soft deep drawing, vacuum packaging, pillow-pack, stand-up pouches, and shrink-wrapping. Finally, injection-molded units are used for packaging of semihard and hard cheeses. Vacuum packaging is primarily used during ripening and not for consumer packages (34), whereas modified atmosphere packaging is extensively applied for retail packages and bulk products for the catering industry.

The cheeses included in this category may be produced by applying different starter cultures—for example, Cheddar produced from a mesophilic O culture,* Havarti cheese produced from a mesophilic DL starter culture,† and Swiss cheese produced from a thermophilic lactic acid starter and propionic bacteria. These cultures, along with their enzymes and so on, produce and use different gases during their metabolism, which should be taken into consideration when tailoring permeability of the packaging materials and when optimizing the gas composition for modified atmosphere packaging. In Swiss cheeses, such as Emmental cheeses, excessive amounts of CO_2 are produced during lactate conversion. Stehle (60) noted that the gas composition in Emmental holes consisted of 95% CO_2 and 5% N_2. The high CO_2 production effectively prevents mold growth, which calls for the use of a highly CO_2 permeable film in order to avoid blowing of the package. In contrast, packaging materials used for Cheddar cheese should be sufficiently impermeable to oxygen to prevent oxidation and mold growth, because only minimal amounts of O_2 are converted to CO_2 in Cheddar. For most materials, the ratio between O_2 and CO_2 permeability is approximately 1:4 (55). However, recently the use of biobased materials such as polylactic acid–based materials has been suggested for cheeses producing high levels of CO_2, because the ratio of O_2/CO_2 apparently is more favorable for such cheeses (61).

Modified atmosphere packaging is extensively used for consumer-packaged cheeses to increase shelf life. Gas mixes containing 10–40% CO_2/60–90% N_2 are recommended for retail packages, whereas 30–100% CO_2/0–70% N_2 is recommended for bulk packages

* Contains only acid-producing strains.
† Contains citrate-fermenting bacteria.

(62). Berne (63) states that the optimum mixture for hard cheeses is 75% CO_2/25% N_2. Oxygen should be kept at a minimum, because keeping oxygen levels may prevent mold growth (71). The minimum partial pressure of O_2 may also be affected by the CO_2 levels present. Furthermore, recent studies revealed that even residual oxygen levels of less than 0.5% were insufficient to avoid photooxidation when products were exposed to light (15,16,18,19). Gases with high levels of CO_2 should be avoided, particularly for sliced and shredded cheeses, in order to reduce packaging collapse around the product during the absorption of CO_2 into the product (64). However, Alves et al. (65) and Eliot et al. (66) found that packaging in high CO_2 concentrations (i.e., 75–100% CO_2) constitutes an interesting alternative for shredded and sliced Mozzarella cheese.

Different O_2 and CO_2 levels are expected to result in changes in microbiota flora with subsequent differences in aroma development. However, documentation in this area is almost nonexistent (17).

Active packaging concepts, which may be suitable for matured cheeses, include oxygen absorbers, carbon dioxide emitters, and slow release of antimicrobial and/or antioxidative compounds from the packaging materials. Additionally, the use of thermoreversible dyes and inks is increasing, with the purpose of boosting sales. Mortensen et al. (15–18) applied oxygen absorbers when packaging semihard cheeses and found that the absorbers were noticeably efficient in obtaining low levels of oxygen and postponing photooxidation. The advent of anaerobic conditions or sensory changes due to low oxygen levels were not evaluated in the study.

Packaging materials used at present include laminates consisting of polypropylene (PP, oriented/OPP), polyamide (PA/Nylon), polyethylene (PE), or polyethylene terephthalate (PET, amorphous/APET or crystalline/CPET). Polyvinylidene chloride, ethylvinyl acetate (EVA), or ethylvinyl alcohol (EVOH) may be used to improve gas barrier properties. Furthermore, aluminum foils are applied and polyvinyl chloride (PVC) is still used in some countries for in-store retail packaging. Packaging materials with low light transmission rates in the critical area, 350–520 nm, may reduce photooxidation considerably (24).

Examples of packaging films include:

- 1 μm laquer/12 μm aluminum/2 μm lacquer (very low gas and water vapor permeability, light protection, block cheese)
- 20 μm coextruded oriented polypropylene/12 μm polyethylene terephthalate/50 μm polyethylene/ethylvinyl alcohol/polyethylene (low gas permeability)
- 15 μm oriented polyamide/60 μm polyethylene (medium gas permeability, low water vapor permeability—for example sliced cheese, non–lactate-fermenting cheeses).
- 20–25 μm coextruded oriented polypropylene/40–60 μm polyethylene (high gas permeability, e.g. lactate-fermenting cheeses).

C. Processed Cheeses

Processed cheeses are prepared by blending and heating natural cheese with emulsifiers to produce a homogeneous paste, which is subsequently packaged and cooled. Product shelf life is extensive (67), and the cheese is most often packaged in materials suitable for hot-filling. Processed cheeses are packed as individual portions or in bulk units. For the individual triangular portions, lacquered aluminum is often used (to prevent corrosion). The influence of different packaging materials (tin cans, polystyrene cups, and low-density polyethylene tubs) and storage temperature was evaluated by Goyal and Babu (68) with tin cans offering the best product protection. To facilitate opening of the portions, an

opening device (red colored strips of either cellulose film or polyester) is generally used (60). The individual packages are assembled into circular paperboard cartons or plastic containers with lids. Larger volumes are packed in glass jars or plastic cups of (for example) polyethylene terephthalate (PET) or polypropylene (PP), polystyrene/ethylvinyl alcohol (PS/EVOH), or aluminum. Because many of these products are warm-packaged, the packaging materials must withstand high temperatures. Recently, Schär and Bosset (69) reviewed quality changes in packaged, processed cheese.

D. Mold- and Smear-Ripened Cheeses

This category is defined as cheeses in which lactic acid fermentation is combined with other ripening processes. The body is neither cooked nor pressed and may contain intenal mold (36). The category includes internally ripened blue-veined cheeses (e.g., Roquefort, Danablu, Stilton, Gorgonzola), surface-ripened white-mold cheeses (e.g., Camembert, Brie), cheeses ripened by red smear bacterial cultures (e.g., Brick, Muenster), and cheeses ripened by both red smear cultures and mold (e.g., Limburger, Taleggio).

In contrast to fresh cheeses, mold cheeses apparently require only limited light protection. Light has no damaging effects on the thick mycelium layer, which also protects the cheeses by reflecting and absorbing the light (36). To the authors' knowledge, light-induced lipid oxidation in mold and smear-ripened cheeses has not yet been reported, which is probably partly ascribable to the rather strong flavor characteristics of these products, which may mask such off-flavors.

Modified atmosphere packaging of mold cheeses is rather complicated because the mold growth must take place at a controlled rate. Packaging at low oxygen levels extends the shelf life, because it slows down respiration of the mold. However, most mold cheeses are sensitive to anaerobic conditions, which leads to decomposition of the mycelium or formation of off-flavors. Hard, blue cheese may be packaged in pure nitrogen in order to prolong shelf life. Carbon dioxide favors the growth of some molds and suppresses the growth of others (48). Hence, CO_2 levels should be tailored to the individual products. Piorgiovanni et al. (70) found that 10% CO_2/90% N_2 resulted in the best sensory scores when packaging Tallegio cheese. Furthermore, the researchers developed mathematical models describing CO_2 development when changing selected packaging variables.

The relative humidity within the package is also a critical factor in controlling the growth and survival of the molds, which implies that the packaging material must also provide an adequate water vapor barrier in addition to ensuring optimal gas permeability conditions.

Mold cheeses are primarily packaged by simply wrapping the material around the product. Flow packaging and deep-drawing are applied for blue cheeses, which are less sensitive to mechanical damage during the packaging process. Many of the soft cheeses are further packaged in wooden boxes, which provide mechanical stability and appeal to the consumers. Perforated foil laminates are used, with the perforation adjusted to allow a certain amount of O_2 ingress and yet prevent dehydration (36). If the materials are too tight, the fungus mycelium will autolyse. Hence, perforation is adapted to suit the individual cheeses. Spot or strip lamination between the individual layers of the packaging films is applied to obtain a more open film for white mold cheeses.

Cellophane is extensively used because it allows ingress of O_2 and sufficient H_2O hydration, preventing fungus autolysis. However, cellophane films are rather expensive and substitutes are increasingly being used, for example packaging films based on polypropylene, which are perforated to obtain the required H_2O barrier. Pearlized polypropylene

(produced by addition of silicium oxide) may be used to obtain materials, that are perceived as "softer" by the consumers.

Packaging of square and triangular cheeses imposes different dead-fold requirements (little resilience) on the materials, which may be met by increased thickness of the kraft paper, by using aluminum foils, and by metalizing the materials. Goat cheeses contain excessive amounts of water, which should disappear during storage. Hot-melt is used when sealing properties are required, whereas paraffin is used when sealing is not required, namely when the product is packaged in cardboard or wooden boxes after having been wrapped in paper or cellulose-based laminates. Blue-veined cheeses seem to be less dependent on the gas permeability of the packaging material; thus, laminates or less open packaging materials are used for blue-veined cheeses (36). Finally, metal cans are used for heat-sterilized Camembert cheeses.

Examples of packaging materials used include:

Cellophane (transparent or white)/32–40 g/m^2 kraft paper/paraffin or hot-melt (Camembert)
Microperforated 15 μm aluminum/cheese varnish (Blue cheese)
20 μm polyproylene/37–45 g/m^2 grease-proof paper (Muenster)
22 μm cellophane/5 μm wax/8 μm aluminum/2 μm lacquer (white and white/blue mold cheeses)

IV. CONCLUSIONS AND PERSPECTIVES

At present, choices of packaging materials and methods are primarily based on practical experience and/or empirical methods. However, it is crucial to commercial viability to target research and development activities on interactions between packaging, the surrounding headspace, and the product. Different types of cheeses call for different packaging concepts; hence, in-depth knowledge is required of the most important quality changes taking place in the various types of cheese in order to individually tailor the packaging solutions. In this respect, incorporating both packaging and product expertise from the onset of the research and development process is vitally important. Furthermore, it is imperative that storage conditions that resemble real-life situations be used.

In order to optimize product/packaging compatibility, materials with improved barrier characteristics should be developed. Optimization may include new areas such as biobased materials, active packaging concepts, and nanocomposite technology.

Solving light-induced oxidation by developing suitable packaging concepts is a major future challenge for the food and packaging industries. This challenge constitutes a significant quality-determining factor during the coming years due to longer store opening hours (increased exposure time) and a general down-gauging of materials (thinner materials, oftentimes with reduced barriers towards light and/or oxygen).

Further research on how packaging interacts with the microbiota is required. The packaging conditions determine which cheese-related organisms will survive or outcompete the others during storage. Thus, improper control of such complex interactions between the microbiota, the cheese, and the packaging conditions lead to selection of unwanted organisms in the cheese or development of off-flavors. However, if the packaging conditions are optimal, cheese ripening may continue at controlled rates in the retail package.

Packaging of sliced and grated cheeses in convenient, transparent, ready-to-serve quanta is a new phenomenon, that imposes requirements on the packaging materials

different from those of block cheese. Additionally, packaging of convenience products partially containing cheese requires optimization and development of new packaging concepts. Research and development efforts in these areas are expected to intensify further in the years to come.

The packaging field is multifaceted and calls for collaboration between different disciplines and application of experimental design and multivariate data analysis. Increased data power and specialized software already facilitate data treatment and modeling of permeability and quality deterioration, which may subsequently reduce product development time and ensure market viability.

Several food producers underestimate the effect of the packaging on quality deterioration. In order to preserve product quality, it is of paramount importance to thoroughly understand and focus on the interactions taking place between the packaging and the product. Only by applying this knowledge for further tailoring of packaging to the individual types of cheeses will the cheese producers secure their cutting-edge positions.

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Cheese Production: Quality Control and Sanitation

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I. INTRODUCTION

The production of cheese is a process of concentrating milk by the interaction of the milk, starter cultures, and, in most cases, rennet. Traditionally, cheese has been produced in small vats, but during the past 3 to 4 decades, processing has become increasingly industrialized. Before industrialization, little attention was directed toward the hygienic aspects of cheesemaking, partly because the batch sizes were small and partly because methods of analysis were not well developed; consumption of cheese would normally cause only a few of disease cases. In recent years, more attention is being given to the hygienic aspects for several reasons: methods for detecting pathogens have improved; more focus on emerging pathogens such as *Listeria* and *E. coli* H7:O157; and the larger batch sizes increase risk to larger numbers of consumers, if pathogens are present. Furthermore, because of the large batches, economic losses will be substantial if the quality is not acceptable. Finally, an unacceptable quality in just a few batches from a producer may lead to loss of market shares. All these reasons have led to a considerable increase in attention on the hygienic aspects of cheesemaking. These aspects include a range of factors such as hygiene, environmental and technological factors, interactions between microorganisms, and the setting up of control systems in order to prevent contamination or at least to inhibit the growth of pathogens.

Complications in doing this arise from the fact that there are many different cheese varieties, such as yellow (with or without surface ripening), fresh, blue-veined, white-molded, and cottage cheese, each with its own risks for the presence or growth of pathogens or spoilage microorganisms. In this chapter, the most important physical, chemical, and microbiological factors required for inhibiting or avoiding pathogens or spoilage microorganisms are described. The creation of a comprehensive control system is discussed.

II. GENERAL ASPECTS OF CHEESE

Cheeses comprise a huge number of varieties and thus the composition also varies. The chemical composition of a cheese results from production under either high or low

acidification, depending on the type of cheese, and the starter culture, which also plays a role in formation of the metabolic profile. As an example, the starter culture is able to form lactic acid as the major component, diacetyl, ethanol, acetic acid, benzoic acid, and bacteriocine (1).

Depending on the type of cheese, the water content varies from very low in Grana cheeses to very high in cottage cheese, and the pH may vary from very low in in blue-veined cheeses and Feta (4.6–4.8) to very high in Queso Fresco (6.2–6.5). As pH varies, so does the lactic acid content. The sodium chloride content varies from very high (4–6%) in some blue-veined cheeses and Feta to very low (0.8–1.0) in cottage cheese. The sodium chloride content, dry matter, and other salts in cheese are responsible for the water activity, which is a very important growth determinant for microorganisms.

Regarding growth of microorganisms on the surface of cheeses, the packaging conditions are very important because the oxygen barrier varies, depending on the packaging material. The choice of packaging material depends on the type of cheese to be packed. Curing times for some cheeses may vary from short (1–6 days) or longer (up to 2 years) time intervals. This factor challenges the hygienic conditions in the curing rooms in relation to the chemical composition of the cheeses. Another challenge in this respect is the variation in temperatures that may occur in curing rooms. The temperature may vary from very high (20–22°C), for example for some Swiss-type cheese varieties, to very low (2–5°C) for fresh cheeses or special varieties. During the curing time, temperature is elevated or lowered depending on the cheese type to be produced. Finally, the addition of nitrate or lysozyme to prevent growth of primarily *Clostridia* is an antimicrobial factor to be considered (2).

From a hygienic point of view, the cheese process in itself is a stabilizing factor. Starter culture is added to the cheese milk at 30°C and, together with the action of rennet, the milk coagulates to form a gel. As pH drops through the formation of lactic acid from the starter culture, the water-binding capacity of the proteins drops. This, together with cutting of the formed gel, separates the milk into cheese and whey. After about 90 min, depending on type of cheese to be produced, the pH has dropped from 6.7 in fresh milk to about 6.0. After this initial cheese process, the cheese mass is pressed and anaerobic conditions are created. After pressing, the cheeses are left to complete acidification to the minimum pH (5.2), typically requiring 24 hr. Most cheese types are then cured in curing rooms at different temperatures, and they may be ripened with or without a surface ripening culture. Some cheeses are packed in different foils in the curing room. Some fresh types of cheeses, however, are packed directly, then stored at 5°C and consumed within a few weeks.

III. ENVIRONMENTAL AND TECHNOLOGICAL FACTORS

A. Organic Acids and pH

The starter culture consists of lactic acid bacteria (LAB), and within 24 hr, the minimum pH is usually achieved. The minimum pH may vary, but in most cheeses it is about 5.2 or lower; for Cheddar pH of 5.0 is normal, and in Feta the pH may be as low as 4.6. The buffer capacity of the cheeses is high due to the high protein content, but the amount of lactic acid formed in the cheeses is also very high, up to 1.5% for some cheese types. This amount of lactic acid, and the relatively low pH, achieves inhibition of many pathogens and spoilage microorganisms, especially gram-negatives. However, the gram-positives will also be inhibited under these conditions. Yeast and molds are only

affected a little by the low pH and high amount of lactic acid. Depending on the type of starter culture, certain amounts of other organic compounds will also be formed (1). When gas-producing mesophilic LAB are used as starters, diacetyl is formed in amounts that are able to cause a little inhibition of pathogens and spoilage microorganisms. Due to the metabolism occurring in the cheeses, the starters will also form acetic acid; up to 250 ppm is normal. This amount is not enough to prevent the growth of pathogens or spoilage microorganisms and has little impact. Other organic compounds such as benzoic acid and ethanol may also have an impact on the growth of pathogens and spoilage microorganisms.

Although the amounts of organic compounds formed are difficult to control, it is easy to control pH and it is important to keep it as low as possible without altering the desirable organoleptic properties of the cheeses.

B. Temperature

Temperature and curing duration are important variables from a technological and hygienic point of view. Although the curing temperature and time may improve the organoleptic properties of the cheese, it may also possibly lead to microbial growth. At 1°C, given the right conditions, *Listeria* is able to grow (3), whereas others such as *Clostridium tyrobutyricum* are not able to grow below 8°C (4). Therefore, it is important to monitor the interaction between the curing temperature and time, in relation to the growth of selected microorganisms, and the cheese's organoleptic properties. From a hygienic point of view, the temperature should be kept as low as possible.

C. NaCl and Water Activity

At a high NaCl content, and/or low water content, many microorganisms are prevented from growing (5). In such cheeses, Staphylococci, *Listeria* and yeast are chief concerns, because they are salt tolerant (6). In fresh cheeses of which the water activity is high and the NaCl content is about 0.8–1.0, the risk of growth is high: these are physiological conditions. It is not possible to lower the NaCl amount because it originates from the milk, and, in many cases, it is not possible to elevate the amount due to changes in the organoleptic properties. In these cases, other means must be used to prevent growth of pathogens and spoilage microorganisms.

D. Nitrate and Lysozyme

Nitrate and lysozyme are often added to cheese milk in order to prevent late blowing from *Clostridium tyrobutyricum* (2–7). In most cases, these additives also inhibit the growth of other microorganisms. But it is worth noting that the activity of the starter may also be slightly inhibited, causing a slower decrease in pH during the fermentation process, resulting in less inhibition of pathogens and spoilage microorganisms during the acidification process.

IV. ANTAGONISTIC/SYMBIOTIC ACTIONS IN CHEESES

For several years, nisin, a bacteriocin produced during fermentation, has been recognized as preservative in a variety of cheeses. Nisin is produced by *Lactococcus lactis* subsp. *lactis*, one of the species used for acidification. The ability to produce nisin is strain dependent.

Nisin can be added to cheese milk or processed cheese as a powder for inhibiting gram-positives. Use of a living nisin-producing *Lactococcus* in cheese production is not widespread because inhibition of the starter culture may be a problem. Other bacteriocins are known (8,9). The starter culture used in the production of surface-ripened cheeses consists of a mixture of yeast, *Brevibacterium linens*, other coryneform bacteria, *Micrococcus*, *Staphylococcus* (primarily *equorum* and *xylosus*), and gram-negatives in limited numbers (10). Bacteriocins from *B. linens* and *Staphylococcus* have been reported, and this is considered to be one way to control *Listeria* on surface-ripened cheeses (11–13). *Enterococcus* sp. has also been reported to produce bacteriocins, and this production may also have an impact on the control of harmful gram-positives on cheeses.

Apart from producing organic inhibitors and bacteriocins, the starter culture may also inhibit other microorganisms by direct competition for substrate. The starter culture ferments lactose into lactic acid/lactate, and thus inhibits the growth of harmful lactose fermenting microorganisms like coliforms or spoilage bacteria (e.g., heterofermentative lactobacilli). Other substrates converted by the starter culture during cheesemaking are citrate and protein fragments, which means that these compounds cannot serve as substrate for pathogens. The formation of lactic acid/lactate will in turn promote the growth of lactate-fermenting microorganisms (e.g., certain *Clostridia*). The best known is *Clostridium tyrobutyricum*, which causes late blowing in cheeses; however, there are means available to prevent this (see Sec. VI.A).

V. HYGIENIC ASPECTS OF EQUIPMENT

A. Bactofugation

Bactofugation is widely used as a means to remove sporeformers from milk; well-functioning bactofugation removes up to 98% of the spores (14). During the autumn and winter seasons, when cows are fed with silage, the spore content of *Clostridium tyrobutyricum* may be as high as 4000 per liter of milk; as few as 10–20 spores per liter may cause late blowing in cheese. With a removal efficincy of 98% by bactofugation, the number of sporeformers remaining is about 80 per liter; thus, bactofugation is not completely effective in preventing late blowing. A relatively new process involving double bactofugation is usually enough to prevent late blowing. Normally, bactofugation is able to remove about 70% of the non-sporeforming flora, but this is far from sufficient removal of the non-sporeforming microorganisms.

B. Microfiltration

A better, but also more expensive, way to remove bacteria in general is microfiltration. Microfiltration will remove about 99.9% or more of the microbial flora present, including sporeformers. By this, the quality of the cheese milk is improved and the risk of the presence of microorganisms will decrease considerably. In cases where it is crucial that special spoilage microorganisms are absent, it is appropriate to perform microfiltration prior to pasteurization.

C. Pasteurization

Pasteurization is the ultimate step for removal of pathogens. Low pasteurization is defined as the combination of time and temperature and is sufficient to kill all vegetative pathogens.

Still, it should be noted that not all microorganisms are killed by low pasteurization. Spores of *Bacillus* and *Clostridium* sp. will survive along with a few spoilage microorganisms such as heterofermentative *Lactobacillus*.

In the pasteurization process, it is important to control the temperature. During pasteurization the temperature will oscillate from the set point, and it is crucial that the lower temperature be above 71.8°C. Controlling this requires an accurate temperature detection system. Such a system should be able to register the temperature rapidly and with high frequency. Another issue in pasteurization is the temperature differences between the components in the pasteurization unit. For example, if the differences in the regenerative system are too big, fouling may occur, leading to lower efficiency in the pasteurization unit. The operation time for the pasteurization unit is also of importance. With the demands for high production efficiency, running times tend to increase, but this is often compromised due to biofilm formation. Finally, of course, the cleaning of the pasteurization unit is important; one must consider the concentration of the cleaning agents and the temperature used.

D. Cheese Vats

The cheese process is normally conducted at 30°C, with a cooking temperature range from 35 to 55°C. These temperatures are the normal interval in which pathogens or spoilage microorganisms are able to grow or survive. It is, therefore, necessary that the cheese vats be maintained in a highly hygienic condition. There should not be any dead ends in the vats, and the interfaces between the cheese vat and pumps, stirring systems, and so on should be secured properly. Finally, it is important that cleaning is easy to perform either as a "cleaning in place" (CIP) system or manually.

E. Brines

In most cases, cheeses are subjected to brine with a NaCl content of about 21%. Direct salting may also be used—for example, in Cheddar and cream cheeses. Due to the high salt content, only a few microorganisms pose a risk; yeast (as spoilage microorganisms), *S. aureus*, and *Listeria monocytogenes* are the only microorganisms of concern. It is also important to note that an infection in the brine leads only to surface contamination, as the cheese at this stage is already formed and the surface has been closed during pressing.

F. Curing and Packaging

There are three methods for curing cheeses: packaged in bags or foil; unprotected on shelves, with surface ripening; and unprotected on shelves, without surface ripening. If the cheeses are packaged, the risk of contamination and/or growth is small. In general, packed and subsequently cured cheeses keep their characteristic low pH, which along with the packaging protects against contamination.

Cheeses that are not packed, and without surface ripening, have a higher risk of contamination. For surface-ripened cheeses the risk of contamination is higher than if they are also packed. The microorganism used for surface ripening will develop into a thick layer and, hence, protect against contamination partly by producing antagonistic compounds such as methanthiol and bacteriocins, and by substrate competition.

G. Distribution and Cheeses on the Market

Obviously the risk of contamination is very low when cheeses are distributed packaged and will only be contaminated if the packaging is damaged. The risk is if pathogenic or spoiling microorganisms are already present in low amounts. They may grow if the cooling chain is broken. This factor is often seen in the cooling desks at the supermarkets, especially, where the temperature often is as high as 15°C, 10°C above the required 5°C; precautions should be taken to keep the temperature at 5°C or below.

VI. IMPORTANT MICROORGANISMS

There are many species of pathogens or spoilage microorganisms to be considered, in and on cheeses. However, many microorganisms are not found in cheese or will not grow during the cheese process. Absence of other microorganisms is controlled by veterinarian authorities in the primary (at the farmhouse) production. Among others *Brucella*, *Mycobacteria*, and *Tuberculosis* are under veterinarian control in most countries.

A. *Clostridia*

Clostridia are widespread in nature and occur in raw milk. Only very few cases of illness due to *C. botulinum* can be attributed to cheese; thus, the major concern is spoilage due to *C. tyrobutyricum*, which causes late blowing of hard or semihard cheeses. Late blowing occurs when the number of *C. tyrobutyricum* in the cheese milk exceeds 10–50 spores per liter and the pH is 5.2 or higher (15). During late blowing, lactate is converted into butyric acid, carbon dioxide, and hydrogen; spoiling is characterized by extreme eye formation, split defects, and off-flavors. Prevention of spores in cheese milk can be achieved, to some extent, by bactofugation (16), but bactofugation is not adequate to prevent late blowing. Double bactofugation or microfiltration is, however, sufficient to prevent late blowing. If it is not possible to bactofugate or microfiltrate, the addition of nitrate or lysozyme is an alternative, but the legal amounts allowed of these compounds may not be sufficient to prevent late blowing. An effective alternative is to cool the cheese down below 8°C, at which temperature the spores will not develop.

B. *E. coli*

Normally, *E. coli* should not occur in cheeses, although 10–1000 *E. coli* per gram can be allowed from time to time, depending on the cheese type. The major concerns are the pathogenic *E. coli* types. These are divided into enterohemorrhagic (EHEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enterotoxigenic (ETEC) (17). They can cause serious disease and have been reported to cause foodborne diseases in at least five outbreaks (18–20). Most concern is with the EHEC *E. coli* H7:O157 that was involved in an outbreak in cheese produced from raw milk. In order to prevent pathogenic *E. coli*, it is crucial to pasteurize the cheese milk. This will assure that the pathogens are not present in the milk, although postcontamination may occur. Good manufacturing practice is normally sufficient to prevent such contamination. If postcontamination does occur, it is important to prevent microbial growth. The activity of the primary starter should be controlled to assure a fast pH drop to below 5.5, which will inhibit *E. coli* growth. If hard

cheeses are produced, the water activity should be held as low as possible, because growth of pathogenic *E. coli* does not occur at a water activity below 0.96. Such conditions are present in some blue-veined cheeses such as Danish Blue and Roquefort.

C. *Salmonella*

As for pathogenic *E. coli*, only a few cheese-related *Salmonella* outbreaks have been reported (21–23). Because *Salmonella* are very heat and salt sensitive, they are not likely to grow in cheese. Thus, prevention of their contamination of cheese milk is crucial. Properly pasteurized milk is sufficient to eliminate *Salmonella*, and the same precautions as described for control of *E. coli* should be taken. Fast acidification and good manufacturing practices along with maintaining low water activity as is possible is usually enough to produce safe *Salmonella*-free cheeses.

D. *Listeria*

Listeria is widespread in nature and can be found in up to 50% of milk samples from raw milk silotanks, depending on geographical and seasonal variations. *Listeria monocytogenes*, a gram-positive pathogen, has caused a few outbreaks of disease. Two of these outbreaks have caused higher rates of mortality (24,25). In 1985, 48 people died due to consumption of a Mexican-style cheese (25). The reason for the contamination was a leak in the pasteurization equipment, resulting in a mixing of raw and pasteurized milk. In the mid 1980s, 34 people died due to consumption of Vacherin Mont d'Or from Switzerland (24).

Listeria is difficult to control due to its relatively high heat stability (D10 at 69°C is about 15 sec). The temperature range for growth is 0–45°C, at pH 4.4–9.5, and up to 10% NaCl. In order to prevent growth, it is important to pasteurize efficiently, which means ensuring that the cheese milk has been heated to at least 72°C for 15 sec. In the above mentioned disease outbreaks, the products had characteristics that favored growth of *L. monocytogenes*. In the Mexican-style cheese, for example, only a weak acidification took place and the NaCl content was low. In the case of the Vacherin Mont d'Or, insufficient hygiene, coupled with a rise in pH and a low NaCl content also favored growth of *L. monocytogenes* (24). It is also worth noting that bacteriocin-producing starters and surface ripening cultures may inhibit the growth of *L. monocytogenes* (12). The infectious dose of *Listeria* is high compared to *Salmonella* and pathogenic *E. coli*, which makes it easier to control in the products. But, on the other hand, *Listeria* is more likely to grow in the final product, depending on the type of cheese, because of its high resistance to low pH, NaCl, and low temperature.

E. *Staphylococcus aureus*

S. aureus is associated with milk because of its close association with cows. It is relatively salt tolerant but sensitive to pH. The infectious dose is high, about 10^5 per milliliter. Disease arises from heat-stable toxins, which means that even though no living *S. aureus* may be detected, the toxins may still be present (26). Thus, it must be assured that the number of *S. aureus* transferred from cow to product does not exceed 10^5/mL, which is assured by a good manufacturing practice on the farms, including ensuring cooling in milk tankers and raw milk silotanks. Because *S. aureus* does not grow below about 8°C, the holding temperature should be held below 8°C, especially if the milk is stored for a long

time in a silotank. *S. aureus* will grow only in cheeses with low acid content or if the cheese surface pH rises during curing. Good hygiene is normally enough to assure either the absence or low numbers of *S. aureus* in or on cheeses.

F. Others

Other microorganisms may cause spoilage or diseases in addition to the ones discussed above. Yeast and heterofermentative lactobacilli may cause gas production or off-flavors in cheeses, although the problem is generally easily solved by cleaning. In both cases, spoilage is due to heavy contamination in the dairy environments, and normally it is easy to control the environments to prevent heavy contamination. There is also the possibility of mold growth, which may be a problem on the surface of cheeses. Formation of molds should be avoided—partly because the damage to the product is severe and partly because formation of molds in some cases leads to formation of mycotoxins. Packaging in a modified atmosphere and in a material that creates a high oxygen barrier will prevent growth of molds. The risk from growth of molds is the formation of mycotoxins, but normally if molds become apparent, the cheese will be destroyed. Mycotoxins are not able to penetrate the entire cheese but are normally located in the outer rind, about 0.5–1.0 cm in depth, depending on the cheese type and water activity.

VII. CONTROL SYSTEMS

In building up a control system, it is important to consider in each step of cheese production, from the farm to the final product, the microorganisms of concern. These steps are called critical control points, and by introducing hazard analysis of critical control points (HACCP), it is possible to introduce a very high safety level in the products. Critical control points are production steps in which some of the physiological or chemical conditions could cause a change conducive for growth of unwanted microorganisms. This means that careful evaluation should be performed if the microorganisms of concern can survive or grow at each control point. The most important parameters to evaluate are temperature; process time at the given temperature; pH and possibility of change in pH; water activity; and addition or formation of inhibitors.

There are different tools for determining whether a control point is critical. One is Predictive Modeling. Predictive Modeling is based on entering a large dataset on the growth of different microorganisms under different conditions into a database system. The growth data on the microorganisms derived from laboratory research, challenge tests, and real product experiences are analyzed statistically and a program is formed that predicts the microorganisms growth at selected temperatures, water activities, pH values, and often under other conditions such as the presence of additives.

Another critical point is the cleaning system. In most dairies, cleaning in place (CIP) is used. Sodium hydroxide with a pH of about 11 and a temperature of 70–80°C is used to wash away most of the milk components from the equipment, followed by flushing with water. Nitric acid at about pH 2 is then used to remove acid-soluble components from the equipment. It is expected that the strength of the CIP and the temperature will drop during the long transportation distances. In both cases, the cleaning efficiency and the direct killing effect on microorganisms will be less. Thus, CIP is an important critical control point. Once the critical control points are established, limits for accepted values are determined and controlled with selected intervals.

VIII. CONCLUDING REMARKS

In order to control the presence and growth of pathogenic microorganisms, it is important to do whatever is possible to prevent their occurrence from farm to cheese product and to ensure that good manufacturing practice is implemented throughout the production. Implementation of HACCP is an excellent tool to control the pathogens or spoilage microorganisms. Raw milk should be of good quality, and its storage should be at low temperatures, especially if storage times are long. Pasteurization is also critical; thus, the pasteurization plant should be under careful control: The temperature must be stable and not below 72°C, the pasteurizer should be cleaned at required intervals, and it must be assured that there are no dysfunctions such as mixing raw and pasteurized milk. The activity of the starter must be high; this will lead to a fast drop in pH, helping to control pathogens. If possible, the temperature should be kep as low as possible and the salt content as high as possible. During the process, hygienic precautions should include good personal hygiene, high water quality used for the production, and adequate cleaning efficiency. It must be ensured that the CIP system be optimal with regard to strength of the sodium hydroxide and acid used, as well as the temperature employed during the cleaning step.

It should be emphasized that foodborne illness outbreaks associated with cheese consumption seldom occur. It is encouraging that considering the huge amount of cheeses consumed worldwide, only a few outbreaks have been documented. One of the reasons for this is that cheese is a well-conserved system, creating a protective chain of hurdles against pathogenic and spoilage microorganisms.

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Meat Fermentation: Principles and Applications

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I. INTRODUCTION

Fermented foods can be defined as products whose specific properties are mainly due to the effects of bacterial metabolism during their manufacture. Although meat products are not the most representative examples of fermented foods, it is clear that microbes contribute to both the conserving and flavoring effects of salt added to meat, a practice dating back to the Neolithicage (e.g., between 5 and 10,000 years ago) (1). Such contribution is evident when the meat surface flora is evenly distributed by chopping, grinding, or comminution of the meat together with the salt. The stuffing of such mixture into a casing produces a sausage, a product first referred to in writing by Homer in 900 B.C. Centuries before Christ, a similar product (*lup-cheong*) was already produced in China; more sugar, however, than salt was added as preservative. Since these beginnings, variable degrees of mixing and comminution of different meats with salt, together with spices, herbs, and other plant material have been applied; this produced a range of products, the stability of which is mainly determined by a combination, varying in relative importance, of

Acidulation, brought about by lactic acid production, and
Lowering of water activity (a_w), brought about by the addition of salt (curing) and drying.

For example, Campbell-Platt (2) discusses 36 whole and 56 comminuted fermented meat products, including an East African sausage made from fat and bones, a hamlike product made by the Greenland Inuits from the eider duck, as well as 33 species of country or raw ham. The contribution of microbial activity in the production of large whole meat products is limited: the meat interior is practically free of microbes, so the specific characteristics of such products are largely determined by remaining muscle proteolytic and lipolytic activity (3), with a limited contribution of bacterial activity to nitrate reduction, color formation, and acceleration of salt introduction by some lactic acid production (4). This introductory discussion will therefore be limited to comminuted products or sau-

sages, subjected to a variable combination of fermentation and drying. The first production of such fermented sausages, as known today, started in Italy in 1730 and spread from there to Germany, Hungary, and other countries, including the United States and Australia. The basic concept of processing involves comminution of muscle and fat tissue with salt, nitrate and/or nitrite, and spices, including eventually sugar, starter cultures, and other additives such as nonmeat proteins. After stuffing the mixture into a casing, the resulting sausage is left to ferment and dry. This ripening often occurs in two consecutive and separate stages, referred to as fermentation and drying, respectively. The lowering of a_w resulting from the presence of salt and drying and the exclusion of O_2 selects for salt-tolerant lactic acid bacteria, producing lactic acid from carbohydrates added and/or present. This lowers pH to final values between 4.5 and 5.5, inducing denaturation of salt-solubilized protein to a gel structure that can be sliced. The adequate (fast) reduction of pH and the lowered a_w ensure both product stability and safety. Once these basic requirements are met, the production technology allows for many but imprecise variations, yielding a variety of very different products. A broad distinction can be made between the following:

Northern-type products (NP), which contain beef and pork and are characterized by relatively short ripening periods, up to about three weeks, involving clearly separated fermentation (about 3 days) and drying periods. Rapid acidulation to final pH values just below 5 followed by product dependent weight losses during drying ensure safety and shelf life. Smoking is applied to add specific flavor (taste and aroma).

Mediterranean or southern-type products (MP), which are predominantly pure pork products and production involves longer ripening periods, up to several months. Fermentation occurs at lower temperatures (ca. 20°C vs $\geq$ 25°C), and acidulation to final pH values above 5 is therefore slower and often not clearly separated from drying. Superficial mold growth is often involved, smoke is not applied (with the exception of Hungarian type salami) and shelf life is mainly determined by drying and lowered water activity.

More detailed and/or official classification of fermented sausages is done in various ways, based on moisture content, moisture/protein ratio, weight loss, water activity a_w, surface treatment, texture, and fat particle size. It has become increasingly clear that the major characteristics of these products are determined by numerous interactions within the trialogue [meat + fat] – [microorganisms] – [processing technology], control of which presents a considerable challenge to standardization and management of quality. Such lack of control and thus, standardization, is apparent from the between and in batch variability observed in the levels of end products of biochemical changes as well as of volatile flavor compounds, showing variation coefficients between 15 and 35% and exceeding 50%, respectively, for both NP and MP in a collaborative European project (5,6). Several aspects of each actor in the trialogue determining variability have been discussed in several recent papers, e.g. on meat enzymes (7), the role of bacteria in flavor development (8), the control of bacteriological safety (9), starter cultures (10), overall sausage metabolism (11), and process engineering (12). This Chapter does not want to add to existing excellent comprehensive literature reviews (4,11) but to briefly summarize some aspects of the subject, emphasizing the importance of interactions for product quality. The text is inspired mainly by the long-term interest of the author's laboratory in an integrated approach to sausage fermentation (13–15) and the role of muscle enzymes (16).

Table 1 Main Production Characteristics for Northern and Mediterranean Types of Fermented Sausages Produced in Belgium

Characteristic	Northern type	Mediterranean type
Diameter (mm)	90	60
Weight	1 kg	0.8 kg
Meat species	Pork/beef, 1/1	Pork only
Lean meat cuts	Pork shoulder + beef	Shoulder
Fat tissue	Pork back fat	Pork back fat
Meat/fat ratio	2/1	2/1
Starter bacteria (CFU)	$+10^7$/g[b]	$+10^7$/g[c]
Additives (g/kg)[d]		
NaCl	30	30
Nanitrite[e]	180	180
Dextrose	7	7
Na-ascorbate	0.9	0.9
Caseinate	11.5	11.5
Spices[e]	7000[f]	7000[f]
Fungal starters	−	+
Particle size	1–2 mm	1–2 mm
Smoking	+	−
Ripening conditions		
Fermentation		
°C/hr	20–26/62	5–24/113
pH after 24 hr	5.70	5.80
3 days	4.68	5.20
% RH/hrs	50–90/62	10–90/89
Drying		
°C/% RH	14/78	14/78
Total ripening time	2 weeks	4 weeks

[a] Unspecified *Lactobacillus*.
[b] 2.10^6 each of *Pediococcus pentosaceous* and *Lactobacillus Sake* as lactic acid bacteria and *Staphylococcus xylosus*, *Kocuria varians* and *Staphylococcus carnosus* as flavor enhancers.
[c] 7.10^5 each of *Pediococcus pentosaceous* and *Lactobacillus Sake* as lactic acid bacteria with 4.10^6 and 2.10^6 of *Staphylococcus xylosus* and *Micrococcus varians* (now properly called *Kocuria varians*) respectively as flavor enhancers.
[d] per kg meat and fat mince.
[e] mg/kg.
[f] Pure black pepper only.
Source: Ref. 6.

II. SAUSAGE MANUFACTURE

Table 1 lists representative conditions for industrial production of both NP and MP, as studied in a recent collaborative research project (6).

A. Ingredients

1. Meat and Fat

The meat to fat ratio is 2 to 1 in most industrial sausage mixes, which contain 50–70% frozen and "tempered" ($\approx -4°C$) lean meat, consisting of pork and beef in equal amounts for NP and exclusively pork for the more valued MP. For pork, ham trimmings, shoulders, and bellies are used, as well as jowls and throats, in proportions determined by the sausage quality level as reflected in more than twofold price differences (F. Vandendriessche, personal communication, 2002). Pork necks are avoided as well as beef head meat, because of risks of antibiotic and Bovine Spongiform Encepalopathy (BSE) contamination, respectively. Only frozen ($\leq -18°C$) pork back and belly fat (excluding less firm sites from the groin and teat line) after removal of rind (lard) and classified according to a visual estimation of fat content (50% vs. 30%, respectively) are used as fat source. Pork rind may be added after cooking. Lard and "sausage meat" account for about 10% and 20% of the pig carcass, respectively (17), and classic selection criteria for these raw materials are mainly based on bacteriological quality and pH (<5.8) and on oxidation status and unsaturation of fat (<12% of polyunsaturated fatty acids, minimal peroxide value) (18, 19). Recent developments, however, report the use of differently specified alternative sources of "meat" and fat as well as the need for more specification for actual meat and fat to improve flavor in the more valued products. Technology for producing fermented sausage from poultry, including ostrich (20), from carp (21), and using olive oil (22) has been proposed. Lyophilized meat can be incorporated to shorten drying time (23), but this may require the lowering of salt addition, unless used after rehydration (24). For the more valued MP, muscle protease and muscle and fat lipase activities are probably important for (spices dependent) sausage flavor development (25) as described for raw ham (3). Relations of pig muscle enzyme activities with both carcass and meat quality have been demonstrated (26,27). Analogous to suggestions made for raw ham production, enzymes and corresponding genes might be considered for specification of raw materials (3) and selection of animals (28) in the production of fermented sausages.

2. Additives

Generally used additives and their ranges of incorporation level include salt (2–4%) containing $NaNO_2$ (80–240 mg/kg added as curing salt containing 0.4–0.6% $NaNO_2$), glucose (0.5–1%), sodium ascorbate or ascorbic acid (0.5–1%), and spices. The use of nitrite is considered essential because of its antibacterial, color forming, antioxidant, and flavoring properties. The use of lower nitrite levels, imposed because of health considerations, requires adaptation of technology—including, for example, minimal fermentation temperatures and oxygen-free chopping (29). For MP, part or all of the nitrite is substituted for by KNO_3, although there is little technological and microbiological necessity for the use of nitrate, and its reported positive effects on color and flavor development may in fact be more related to the accompanying lower nitrite levels (4,30). Both the amounts and the nature of carbohydrates have been related to the rate and extent of acidulation (31), but only the use of lactose clearly results in lowering both rate

and extent of acidulation (32), whereas its residual presence may improve sensory quality (33). Optimal levels of glucose (dextrose) are reported to be 0.3% and 0.7% for MP and NP, respectively (34). Both ascorbate and ascorbic acid are used to improve stability of the red nitrosylated pigment, an effect closely associated with the prevention of lipid oxidation. Both effects may also associated with the presence of antioxidants such as BHT and vitamin E in the raw materials, due to their use in animal feeding. However, although protective effects on lipid oxidation and color stability of supranutritional dosing of animal feeds with γ-tocopherol are clear for beef and, less so, for fresh and cured pork (35), it would seem that it cannot prevent sausage color deterioration due to incorporation of polyunsaturated fat (36). Ground pepper (0.2–0.3%) is usually present in all types of sausages and, especially, MP may contain higher levels (1–3%) of other spices such as paprika and garlic that were shown to be effective antioxidants, comparable to ascorbate (37). Besides yielding flavor compounds, spices may stimulate lactic acid bacterial activity (e.g., by supplying manganese [54]) and decontamination should be considered (38). Apart from these "classic" additives, additional aditives are sometimes used for NP: phosphates (0.5%) to improve stability against oxidation (39), glucono-δ-lactone (GdL, 0.5%) to ascertain fast but chemical acidulation with generally negative effects on flavor development, and vegetable proteins (mainly soya isolate) that may also accelerate fermentation (40).

3. Starter Cultures

Starter bacteria were first introduced in the United States to ensure rapid fermentation. In Europe, lower fermentation temperatures were/are more common and, certainly in MP, rapid fermentation results in color and flavor defects. In order to avoid the latter, micrococci were introduced as starters by Nïvaara (41). Although excellent fermented sausages can be produced without the addition of starter cultures or re-inoculation with finished sausages, the majority of fermented sausage produced in Europe nowadays makes use of "combined" starter inoculation ($1–2.10^6$/g) as frozen cultures of both lactobacilli and Micrococcaceae to ensure rapid acidulation and optimal flavor development, respectively (4). Their desired properties have been discussed at length [e.g., (42)] and obviously include lag time and rate of lactic acid production for lactobacilli and the resistance of Micrococcaceae to an acid environment. Inhibition of the latter by rapid acidulation was indeed found to impair the typical flavor of MP (43). For flavor development, lipolytic and proteolytic activities have long been emphasized. It is now realized, however, that muscle and fat tissue enzymes are by far the more important actors in this respect and more important selection criteria for flavor producing starters such as *S. carnosus* may be their potential for leucine degradation to 3-methyl butanal and for protection of polyunsaturated fatty acids against oxidation (5,8). Attention has also been directed recently to biogenic amine (44) and bacteriocin (45) production by starter bacteria. These aspects are considered of less importance, because raw material quality and processing appear to be the main factor controlling amine production (46), and bacteriocin effects are bound to be limited (10). Bacterocin production by *L. sakei* strains, isolated from fermented sausages, may however contribute to safety, without inhibition of Micrococcaceae and, thus, of flavor development (46,47). Besides bacteria, yeasts and fungi are used to a limited extent in the production of MP. *Dabaryomyces hansenii* and *Penicillum nalgiovense* are the major species sold as as starters for MP production (19). They are both aerobic organisms and thus situated at the periphery and/or surface of the sausage, where they oxidize lactic acid and produce ammonia. Their contribution to flavor development is less

clear; it may involve fatty acid oxidation to methyl-ketones, and the strongest argument for the use of fungal starters may be the prevention of growth of mycotoxin-producing fungi (19).

B. Processing

1. Comminution or Chopping

Industrial processing generally uses the cutter, involving a rapidly rotating set of knives (1–3.10^3 rpm) producing a batter in a slowly rotating bowl (10–20 rpm) within less than 5 min. The relative speeds of rotation of bowl and knives as well the sequence of addition of raw materials and additives determine fat particle size (1–25 mm^2) and are optimized to minimize both damage to the fat tissue added and increase of the batter temperature ($\leq -2°C$). Because air bubbles in the batter and oxygen may interfere with drying and color development, respectively, chopping is best carried out under vacuum. The use of blunt rather than sharp knives is to be preferred for good texture (48). For traditional preparation of MP, often characterized by larger particle sizes, a meat grinder rather than a cutter may be used.

2. Stuffing

Traditional methods using nitrate may still incorporate a "pan curing" phase (49), leaving the batter 24 hr at low temperatures before stuffing to allow for optimal color development. In most industrial processes, however, vacuum-filling devices are used to immediately stuff the batter into natural or man-made collagen or cellulose-based casings, permeable to water and air. The size of sausage diameter (e.g., 2–15 cm) is related positively to the relative importance of fermentation (pH) versus drying (a_w) for stability, and a small diameter of collagen casing is required to ensure sufficient oxygen supply for full development of the mold aroma in MP (4,50).

3. Fermentation and Drying

For industrial production of NP, fermentation and drying are usually carried out in separate rooms. Representative temperature/time/relative humidity (RH) combinations for NP and MP, respectively, are shown in Table 1. It is recommended to have air RH values not more than 0.10 points below the associated a_w values of the sausage, to prevent case hardening. When pH is less than 5.3, a steeper RH gradient may be applied. Recommended air speeds are approximately 0.1 m/sec, and a back-and-forth shifting of RH between 80% and 88% during drying is recommended (34). Controlled fermentation and ripening in air-conditioned surroundings consumes considerable amounts of energy and alternative methods, involving the use of fresh air, have been proposed, inspired by the traditional methods for MP, adapted to local climatic conditions (51).

4. Smoking

At the end of the fermentation period, NP are subjected to smoke, generated by controlled combustion of wood (300–600°C) to minimize the production of polycyclic hydrocarbons. Smoke contributes to antimicrobial and antioxidant effects, besides generating specific flavor and color components. Smoking is not used in the production of MP, except for Hungarian and Romanian products, where a light smoking period precedes fermentation (34).

III. THE NATURE AND DYNAMICS OF RIPENING

It has been repeatedly confirmed that safety, stability, and sensory quality of fermented sausages are the result of a complex and interacting set of microbiological, physical, and (bio-)chemical changes (4,11,13,14). Within this complexity, most attention has been directed to the role of bacteria in relation to both safety and flavor: a "Web of Science" search for "fermented sausage" in April 2002 shows 194 papers published since 1977 (0.00075% of all documents !), 100 of which were published since 1997, containing 62 dealing exclusively with the behavior of desirable or undesirable microorganisms. Safety has been the subject of excellent reviews describing the "hurdle concept" (34) as well as focusing on the potential of bacteriocinogenic lactic acid bacteria (52) and will not be further discussed here. Besides an adequate redox-potential and the presence of nitrite and competitive desirable bacteria, major hurdles ensuring bacteriological safety are considered to be either the rate and extent of acidulation (10) or the extent of drying (lowered a_w) (9) for NP and MP, respectively.

A. The Dynamics of Acidulation and Drying

Both the rates and extents of acidulation and drying can be quantified using simple exponential models (13) allowing demonstration of effects of processing factors such as the use of back slopping and starter cultures, sausage diameter, as well as the use of additives such as spices and soy protein (13,53–55). The same models allow for the quantitative evaluation of the relations between rates of acidulation and those of drying and texture development (19). Predicted changes of DM content are assumed to be determined by water loss (drying) only, and thus can be used to predict weight losses (55), for the calculation of empirical or "effective" diffusion rates (13,56) and, together with data for salt content, for the prediction of a_w values with acceptable precision (57,58).

B. The Nature and Dynamics of Sausage Metabolism

The carbohydrate, protein, and lipid fractions of the sausage are subject to changes brought about by chemical as well as biochemical changes, and the latter are mediated by microbial as well as by muscle and fat tissue enzymes. Multifactorial effects involving raw materials, additives, and starter cultures as well as processing determine the relative intensities of these changes, mainly reflected in the rate and extent of acidulation and the development of sensory quality characteristics.

1. Sausage Metabolism and Acidulation

It is now clear that acidulation of the sausage matrix is induced by the interaction of salt-solubilized muscle proteins with both lactic acid and ammonia formed during fermentation (33,59). Molar amounts of lactate and ammonia present (59) as well as produced (54) during sausage ripening have indeed been related to pH and pH changes, respectively. Lactate is mainly produced from (added) carbohydrates but may also be formed during microbial fermentation of glycerol, liberated in lipolysis, and, together with ammonia, from fermentation of amino acids. Besides lactic acid, variable amounts of acetic acid are produced, determined by the nature of the bacteria and their metabolism, both affected by the processing conditions. Finally, considerable amounts of oxygen are consumed during "fermentation," therefore better referred to as metabolism (60). This overall "sausage

metabolism" affects the relationship between the amounts of carbohydrate "fermented" and lactate produced and has been described in a simplified stoichiometric model (61,14), represented in Fig. 1.

Such a model ignores the complexity of bacterial amino acid metabolism (11), representing it by either deamination to ammonia or decarboxylation to amines. Nevertheless, it has been supported by experimental data, within experimental error (60). Together with simple exponential analytical models, stoichiometry can be used to characterize the respective rates and relative importance of carbohydrate and protein oxidation (respiration) and fermentation, as well as the nature of fermentation (lactate/ acetate) (14). In contrast to the kinetic and analytical models, referred to under Sec III.A, models for metabolism are mechanistic, require more analytical data, and are therefore better suited for comprehensive rather than predicting purposes (62). For example, they have been used to illustrate effects of the processing factors mentioned earlier (Sec. III.A) as well as chopping intensity (63) on the relative importance of respiration and protein fermentation. Table 2 shows that in a series of experiments characterizing ripening metabolism of NP, carbohydrate disappearance accounted for between 82% and 94% of pyruvate equivalents metabolized.

An increase of diameter was shown to increase the relative contribution of protein fermentation as well as the accumulation of free amino acids, buffering the increased rate of lactate production. The relative importance of protein (amino acid) fermentation with ammonia production was lowered by addition of active lactic acid bacteria. When the latter were added during backslopping, however, or as isolates from fermented sausages, proteolytic activity is apparent—an important effect for flavor, as recently confirmed (47).

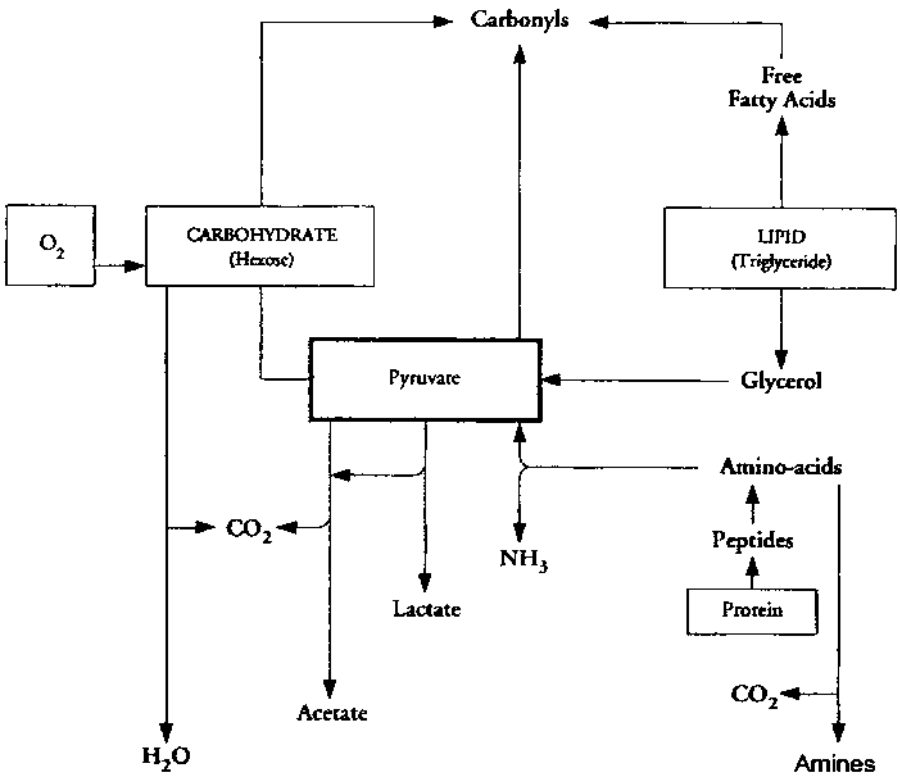

Figure 1 Reaction scheme underlying the stoichiometric model of sausage metabolism.

Table 2 Effect of Processing Conditions on the Pattern of Sausage Metabolism During Ripening of NP (21 days) (% of Pyruvate Equivalents Derived from Carbohydrate and Protein and Utilized in Fermentation to Lactate and Acetate and in Respiration.)

	Added starters[a]			Sausage diameter[b]		Chopping time[c] (min)			
Effect of	None	S1	S2	47 mm	90 mm	4.5		7	
Metabolism (%)						(1)	(2)	(1)	(2)
of:									
Carbohydrate	82	86	91	93	92	87	89	89	88
Protein	18	14	9	7	8	13	11	11	12
to:									
Lactate	54	57	86	60	78	73	83	69	77
Acetate	16	7	7	7	6	6	10	6	10
Respiration	30	35	7	33	16	21	7	25	13

[a] Data from Ref. 53: starters used were fermented sausage, e.g. back slopping (S1) or a lyophilyzed culture of bacteria (S2).
[b] Data from Ref. 13.
[c] Data from Ref. 63: results of two experiments are shown involving longer chopping with salt (1) or pork back fat (2) respectively.

Inhibitory effects on deamination activities of both rates and extent of acidulation, more dramatic with larger diameters, associated with lower rates of drying have been confirmed in recent work (Demeyer http://fltbwww.rug.ac.be/animalproduction). Protein fermentation seems to be associated with the importance of acetate in the recovery of pyruvate equivalents. The most striking finding, however, is the large variability in respired substrate: between 7% and 43% of pyruvate equivalents metabolized. These calculated values obviously reflect an enormous variability in oxygen consumption, which may involve chemical oxidations apart from respiration. Factors determining variation are probably related to processing characteristics such as sausage diameter and chopping (Table 2), but variability in factors such as vacuum stuffing, gaseous exchanges during ripening, and respiratory activity of bacteria and molds may be more important.

2. Sausage Metabolism and Sensory Quality

Estimates of the nature of sausage metabolism, as discussed above, allow better comprehension of the mechanisms determining both rate and extent of acidulation. It is however, clear, that the generalized pathways used in such estimates cover and/or accompany a set of detailed (bio)chemical reactions and end products affecting the development of sensory quality. Flavor is considered to be the more important sensory characteristic, determining repeat purchase by the consumer, whereas texture and color determine initial purchase and rejection (64).

a. Texture. Texture, which can be measured as the force necessary to penetrate the sausage surface or interior (sausage slice) under standardized conditions (e.g., hardness) is determined by two processes, occurring consecutively in NP and simultaneously in MP:

Gel formation due to acidulation. During chopping, myofibrillar structures are degraded (65) and myofibrillar proteins are solubilized into a sol—a network of filamentous aggregations of myosine molecules, whose dimensions and for-

mation depend on factors such as pH and NaCl concentration that determine the relative rates of filament formation and aggregation (66). Acidulation induces coagulation—the conversion of a sol into a gel by intensification of aggregations, associated with the release of water and the formation of a matrix surrounding fat and connective tissue particles. The pH necessary for coagulation increases with increasing salt concentration and is 5.3 for salt concentrations between 2% and 3% (67)

Drying. After gel formation, hardness is further increased because of loss of water, determined by diffusion-limited water transport.

The rate and extent of pH decline in the sausage, itself a reflection of overall sausage metabolism, determines both processes. During fermentation, muscle cathepsin D is activated by the decrease in pH and degrades sausage myosin (68,69). It is known that such damage lowers the strength of heat-coagulated myosin gels (16), and negative effects on texture of sausage proteolytic activity (70) and of increased myosin degradation because of added proteases (71) have indeed been reported. It is therefore clear that acidulation during fermentation induces two opposing effects on texture development: coagulation of the myosin sol into a gel as well as accelerating proteolytic cleaving of myosin molecules, lowering their contribution to gel strength, and, possibly, to water retention within the gel. Differences in gel structure and its water retention because of different relative rates of acid-induced coagulation and proteolysis may explain the positive relationship found between initial rates of acidulation and texture development during drying. The use of PSE pork (72), spices (54), starter organisms (13), and soy protein (40) increase rates of acidulation and drying and thus of texture development. For obvious reasons, an increase in sausage diameter decreases rate of drying and, thus, rate of hardness development (73). Also, however, the rate of pH decline is lowered because of an increasing contribution of proteolytic processes to metabolism (14,74).

b. Color. The stable red color of fermented sausage is due to nitrosylation and subsequent acid-induced denaturation of myoglobin. In MP, formation of the nitrosylating NO occurs after bacterial reduction of added nitrate to nitrite, a process generally attributed to Micrococceae. Its inhibition by pH values below 5.2 hampers the use of lactic acid bacterial starters to ensure rapid acidulation. In NP, characterized by rapid acidulation, nitrite is the additive ensuring color development. However, upon addition it acts as a very reactive oxidant for myoglobin: it is reduced to NO during chopping with an immediate gray discoloration of the batter, due to (nitrosylated) metmyoglobin formation. The rates of both this initial oxidation and the subsequent reduction and denaturation to the red nitrosylated myochromogen during ripening, as well as the stability to subsequent oxidation of the color formed, are determined by a complex set of factors, including the amounts of nitrite used, the rate of pH drop during fermentation, the use of antioxidant additives and the antioxidant activities of the meat and starter bacteria used. It would seem that for the Northern ripening process, the use of sodium ascorbate (e.g., 600 ppm) with minimal amounts of sodium nitrite (e.g., 150 ppm) is sufficient to obtain an acceptable color stability also reflected in a low redox potential (30) and minimal lipid oxidation (75). These conditions are promoted by the use of starter organisms with antioxidant activities (catalase, superoxide dismutase, and/or nitrate reductase activities in Micrococcaceae) also contributing to flavor development (76) and/or low hydrogen peroxide–producing activity (lactic acid bacteria). Net peroxide production is low at lower rates of acidulation (4), and minimal oxidation during sausage metabolism because of a low redox potential may be reflected in its high potential of oxygen removal/consumption (Torfs and Demeyer, in preparation).

c. Flavor. The simultaneous confrontation of the consumer with the texture, taste, and smell of the product during chewing creates his or her impression of flavor. Aroma (smell, odor), determined by volatile compounds, is considered to be the most important component because of the very high sensitivity of the nasal receptors. It is often considered separately from taste, which is determined by nonvolatiles sensed by the receptors predominantly situated on the tongue. One should, however, be conscious of a "taste-olfaction integration" of senses (77), also apparent from the aroma enhancement due to the glutamate-umami taste. Peptides in fermented sausages may have a similar effect. In this respect, it may be significant that fermented sausages were found to be better distinguished by taste than by odor for the descriptor "dry sausage" (78).

Volatile aroma compounds. More than 200 chemical compounds have been identified by gas chromatography–mass spectrometry or gas chromatography–olfactometry in volatiles present in the sausage "head space" or isolated by steam distillation (5,19). Not all compounds in such "spectra" are of sensory relevance, and the majority are derived from spices and smoking (NP). A limited group of compounds, thought to be responsible for the specific "fermented sausage" flavor, however, consist of:

Compounds considered to be derived from carbohydrate metabolism represented by acetic, propionic and butyric acids, acetaldehyde, diacetyl, and acetoin.

Compounds considered to be derived from protein metabolism, mainly represented by branched aldehydes and the corresponding acids and alcohols.

Compounds derived from lipid degradation, mainly represented by methyl ketones, produced by microbial β-oxidation. Chemical autooxidation of unsaturated fatty acids produces a whole range of volatile carbonyl compounds, such as hexanal, contributing to the rancid notes and as such important for the overall flavor.

These compounds have been clearly associated with sensory descriptors such as maturity and salami and their relative importance, as well as that of esters, is increased by the Mediterranean low-temperature and long-time-ripening process with use of staphylococci as starter organisms (79). Higher temperatures and the use of pediococci as lactic acid–producing starters promote the production of dairy-related volatiles such as diacetyl and accelerate the rate of pH drop, inhibiting Staphylococci (5).

Nonvolatile taste compounds. In contrast to MP, an "acid" taste is often sought for in NP and is positively correlated with the contents of D-lactate and acetate (61,80). The extent of proteolysis, as reflected in the levels of low-molecular-weight peptides and free amino acids is clearly correlated with sensory analysis (14), specifically in relation to mold growth (10), and it is known that peptides affect taste rather than aroma, as shown for raw ham (81). Preliminary data (15) have associated small peptide (<500 D) and amino acid containing fractions isolated by gel permeation chromatography with sensory descriptors such as salami and bouillon, in analogy with the known importance of such fractions for raw ham flavor (82). Also, the nonprotein nitrogen fraction will affect sausage pH (59) and may thus affect liberation of aroma determining acid compounds during chewing (83). ATP metabolites such as IMP and hypoxanthine contribute to taste, whereas free higher fatty acids are generally considered of less importance (64).

The relative importance of the different flavor compounds is determined by interactions between muscle and microbial metabolism as well as chemical reactions. The use of antibiotics and paucibacterial meat incubations has clearly established that initial proteolytic changes mainly involve myosin and actin degradation through the action of cathepsin D–like enzymes. The contribution of bacteria in further endo- and, mainly, exoproteolytic

changes increases down to ammonia production, the end of the proteolytic chain. Mediterranean, low-temperature ripening lowers rate of pH drop and thus cathepsin D activity and initial protein degradation, but further proteolysis is not affected. Paucibacterial meat incubations demonstrate free amino acid production by meat enzymes.

In similar experiments, it was clearly demonstrated that endogenous lipases are by far the mainly responsible enzymes for the liberation of free fatty acids during ripening, with preferential release of polyunsaturated fatty acids, both because of the more important phopholipase activity on muscle membrane phospholipids and the specificity of fat cell lipases. The importance of lipolysis for lipid oxidation, and thus flavor, remains unclear but a promoting effect is often assumed (15). Our laboratory has participated in studies on the impact of processing and of bacteria on the production of volatiles important for flavor in meat and meat products (6,15,84), using standardized methodology, and our results suggest that the major contribution of bacteria to dry sausage ripening may be related more to lowering of lipid oxidation than to amino acid fermentation. Anyway, both amino acid fermentation and antioxidant characteristics are probably more important selection criteria for flavor-enhancing starter bacteria than their lipolytic and proteolytic properties. It is indeed now generally accepted that initial proteolytic and lipolytic changes during dry sausage ripening are brought about by enzymatic activity of the raw materials, rather than from microorganisms (10). It is known that such activity in muscle shows considerable variation, related to anatomical location, gender, animal age, and postmortem rate of pH drop (26,27,85). The effects of such metabolic variability on flavor development in relation to bacterial activity should be further investigated.

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21
Dry-Cured Ham

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I. INTRODUCTION

The origin of dry-cured ham is lost in ancient times, when humans used salting as a useful preservation tool for times of scarcity. The evolution of this product has followed a traditional route over the centuries, with oral transmission of the techniques involved from generation to generation but it was produced very empirically, with a rather limited knowledge of the process technology (1,2). In the last decades of the 20th century, rapid advances in the scientific knowledge of the chemistry, biochemistry, and microbiology involved in the process were made (3); this knowledge prompted successful developments in technology and significant progress in quality standardization.

The wide variety of processing technologies (with important variations in the conditions for drying, ripening, smoking, etc.) as well as the important influence of the hams used as raw material (genetic type, feed, rearing system, etc.) make for important variations in quality, especially in sensory characteristics. Main types of hams and the most important processing technologies are described in this chapter.

II. TYPES OF PRODUCTS

Some of the most important and well-known hams are listed in Table 1. Iberian hams are produced in the southwest region of Spain by a long process that usually takes 2–3 years and gives an unique typical flavor. Hams originate from autochthonous heavy pigs, grown in extensive system and fattened with acorns (2). Similarly, Corsican hams take a long time (18 months) to produce in Corsica (France) and are made from autochthonous heavy (around 150 kg live weight) pigs grown in extensive system and fattened with chestnuts, although the output is restricted due to the low number of pigs.

Hams from certain crossbreeds of white pigs constitute the raw material for Spanish Serrano, Italian San Danielle, and French Bayonne dry-cured hams. These pigs are slaughtered at 110 kg live weight whereas those used for Italian Parma hams are heavier (150 kg live weight). Dry-cured hams may receive different labels in the European Union (EU) area, such as Protected Designation of Origin, Protected Geographical Indication, or Traditional Speciality Guaranteed, depending on the specific region and particular regulations

Table 1 Main Characteristics of Dry-Cured Hams Worldwide

Dry-cured ham	Country of origin	Approx. length of process (months)	Smoking
Iberian	Spain	24–36	No
Serrano	Spain	9–18	No
Bayonne	France	9–12	No
Corsican	France	24	No
Parma	Italy	12–18	No
San Danielle	Italy	9–18	No
Katenschinken	Germany	3–5	Yes
Westphalia	Germany	3–5	Yes
Country-style	USA	3–9	Yes
Sauna	Finland	2–4	Yes
Ching Hua	China	3–6	Yes
Yunnan	China	3–6	Yes

Source: Ref. 3.

(e.g., type of crossbreeds, type of feed, slaughter age, processing technology). These hams are controlled by consortiums, such as the Parma Consortium or the Serrano Foundation, that verify the fulfillment of the specific requirements. All these hams can be and require eaten no further smoking or cooking.

Country-style ham is produced in the United States, particularly in Kentucky and Virginia. Hams are salted, dried for at least 70 days or even longer for better flavor development, and then smoked. The traditional German Westphalian ham, the German cold smoked ham (Katenschinken), and the Finnish "sauna" hams are dry-salted, left for a few weeks in vats or wooden barrels, and then smoked (4). Hams are also produced in other areas such as China, where typical hams like Ching Hua or Yunnan enjoy great acceptance (5).

III. PROCESSING TECHNOLOGY

Traditionally, pigs were reared at home and slaughtered by the end of November or early December so that hams could be salted and then left for salt diffusion during the coldest months. During the spring and summer, hams were ripened and dried, becoming ready for consumption by autumn (almost one year of total process). The production sites were usually located in the mountains, with cool and dry weather conditions favoring this process. The windows of the rooms were opened or closed depending on visual and tactile assessment by an experienced operator. Of course, this method was transmitted from fathers to sons, but the subjective assessment resulted in a great variability in the final quality. Today, most modern factories use computer-controlled drying chambers that allow a full control of air speed, temperature, and relative humidity. The final quality depends on the length of the process because time is needed for the enzymatic and chemical development of flavor, as will be discussed later. In general, the process is as schematized in Fig. 1 and consists of the following stages.

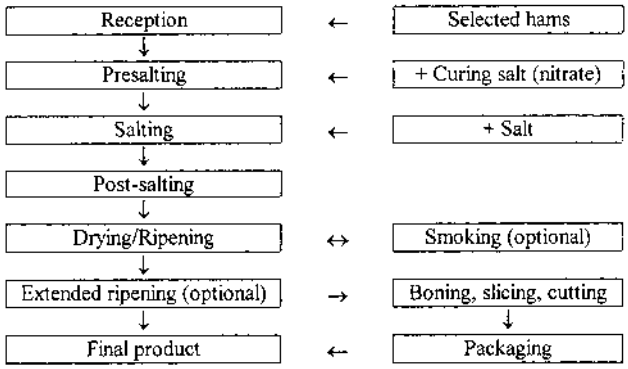

Figure 1 Process flow diagram for the processing of dry-cured hams.

A. Reception

Pork legs are classified when they arrive at the factory in order to facilitate their correct processing. This classification depends on the particular area but is usually based on ham weight, pH, and fat thickness (3). The composition of fatty acids in the fat mainly depends on the animals's feed (6) and, to a minor degree on the crossbreed used (7) but is of extreme importance for correct flavor development. Depending on the composition of certain polyunsaturated fatty acids, hams may develop an adequate flavor or may experience undesirable oxidation and develop rancid off-flavors. Fat may be controlled through the iodine index (as an indicator of unsaturation) and the acid index (as an indicator of freshness). The exudative hams, with a condition known as pale, soft, and exudative (PSE), have a low water-binding capacity and may sustain important weight losses, substantially higher than normal hams (8). In addition, PSE hams have a pale color and a wetted surface that facilitates the dissolution and penetration of the added salt but, on the other hand, results in an excessively salty taste. Other groups of hams, those having high ultimate pH and known as dark firm, and dry (DFD), must be rejected in order to avoid microbial contamination.

Although the modern meat industry uses standard pigs, hams produced from older pigs usually are of better quality due to the higher amount of myoglobin (improved color) and different enzyme profile (better flavor profile) (9,10).

The skin is partially removed, leaving an area where salt will penetrate and water will evaporate. Hams are then registered to facilitate traceability, subjected to pressing rollers for bleeding, and left for 1 or 2 days under refrigerated storage (2–4°C) to reach a uniform temperature. Frozen hams are allowed to thaw till a temperature of about −4°C is reached inside the ham.

B. Presalting

This is a short stage during which nitrate is added to the hams in the form of a curing salt (sodium chloride with 4% potassium nitrate) for a few minutes within a rotary drum (i.e., Spanish Serrano hams). The curing salt may be directly applied in the salting stage (i.e., for French and country-style hams). Nitrate and/or nitrite are used as protective agents against botulism (11). Nitrate is reduced to nitrate by the action of nitrate reductase, a

bacterial enzyme present in the natural flora (i.e., Micrococcaceae) of ham. This reduction is slow due to the low bacterial counts. Further formation of nitric oxide is achieved at slightly acid pH, as found into the ham and favored by curing adjuncts, such as ascorbic or erythorbic acids, that act as reducing substances. The maximum amount allowed in the European Union is 150 ppm potassium nitrate or 300 ppm for combination of potassium nitrate + sodium nitrite, and in the United States 156 ppm sodium nitrite (1/4 ounce per 100 pounds of meat). In some cases, the use of nitrate and/or nitrite is banned (i.e., Italian Parma ham).

C. Salting

Salt inhibits the growth of spoilage microorganisms by reducing the a_w; it also imparts a characteristic salty taste and increases the solubility of myofibrillar proteins. The main objective of the salting stage is to supply the necessary amount of salt to the outer surface of the hams. Absorbed salt is then slowly diffused through the whole piece during the post-salting stage. The amount of salt may be tightly controlled, on a weight basis, allowing time enough for its penetration into the piece (exact salt supply). So, hams are weighed one by one and the exact amount of salt per kilogram of ham is added on the lean surface. For instance, Parma hams receive 20–30 g medium-grain salt per kilogram on the lean surface and 10–20 g of wet salt per kilogram on the skin (12). Then, salt is hand-rubbed and left to be absorbed into the ham (14–21 days, depending on size).

In other cases, the amount of salt is undetermined but time of salting is strictly controlled. Hams are entirely surrounded by rough sea salt or refined mineral salt and then placed by layers into stainless steel bins with holes for the elimination of drippings. Salt may be rubbed onto the lean surface, and the hams are placed on shelves. This stage may last up to 13 days under refrigeration with 3–4% weight losses. In some cases, hams are salted again. Once the salting stage is finished, the excess salt is removed by brushing and water rinsing.

D. Post-Salting or Resting

The main objective of this stage is to achieve salt equalization through the entire piece. The required time may vary between 40 and 60 days, depending on many variables such as the size of the ham, pH, amount of fat, and conditions in the chamber. The relative humidity in the chamber is progressively reduced with time, and the typical weight losses are around 4–6%.

E. Smoking

The use of smoke is one of the oldest preservation technologies, and it is used for short-term processed hams like American country-style or German Westphalia ham. The use of smoking is typical in areas where drying was originally more difficult (i.e., Northern countries) and gives a particular flavor to the hams. The smoke compounds also protect hams against molds or yeasts growth due to their bactericidal effects.

F. Ripening-Drying

Hams are placed into modern computer-controlled drying chambers; some may contain up to 30,000 hams per chamber. Temperature, relative humidity, and air speed must be as

homogeneous as possible and are carefully controlled and registered. Each type of ham has a specific set of variables along time of processing. For instance, Spanish hams are subjected to a progressive slow increase in temperature whereas French hams are heated to 22–26°C just after the post-salting stage. In all cases, these conditions allow the action of the endogenous enzymes, as will be described later. The length of the process depends on the type of ham (pH, size, amount of intramuscular fat, etc.) and drying conditions. The final expected weight loss (around 32–36%) is usually achieved within 6 to 9 months. Then, hams are covered with a layer of lard to avoid further dehydration and prevent any growth of molds and/or yeasts on the outer surface. Hams quality is monitored through a sniff test consisting of the insertion of a small probe in a specific area of the ham prone to spoilage and immediately smelled by an expert for detection of any off-flavor (12). The rapid development of commercial electronic noses and probes to get an objective assessment of flavor quality has led to their increased use for quality classification of hams (13).

G. Extended Ripening

Hams of high quality are further ripened in cellars for several months under mild conditions in order to get a full, rich flavor development. This is the case with Iberian hams, which may undergo 24 to 30 months of total processing time.

H. Final Product

Hams may be sold either as an entire piece (usually those of higher quality) or boned. Commercial distribution of sliced ham in vacuum-packages or under controlled atmosphere is increasing very fast. Boned hams are usually vacuum-packaged and distributed through retailers for final cutting into pieces or slices (see a slice in Fig. 2). Hams are sliced by retailers or directly by consumers at home.

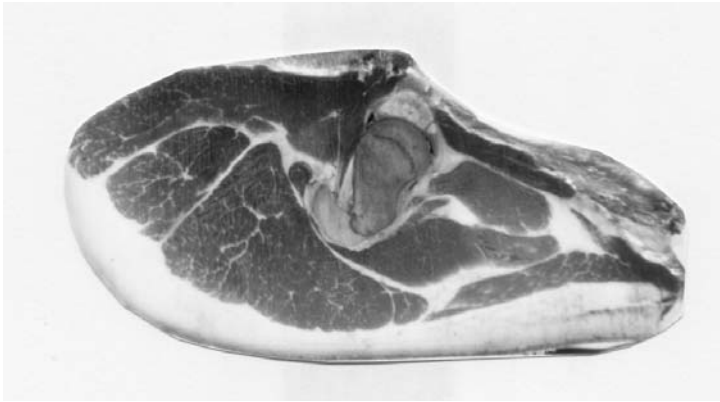

Figure 2 A cross-section of a typical dry-cured ham.

IV. MICROBIAL EVOLUTION

The increased concentration of salt and progressive reduction in water activity constitute limiting factors for microbial growth (14). In fact, low bacterial counts have been found inside the hams (15). Some species of the Kocuria family with nitrate reductase activity are present in ham. Some microorganisms, such as *P. pentosaceus* and *S. xylosus*, which are present in the natural flora of ham, have been studied for their enzyme activity but no significant endoprotease activity was detected, only a minor exopeptidase activity (16) although *S. xylosus* also showed an important nitrate reductase activity. The enzyme profile of some lactic acid bacteria has been also studied, because they could be used as microbial starters to accelerate the process. In fact, *L. sakei*, *L. curvatus*, *L. casei*, and *L. plantarum* have shown good endo- and exoproteolytic activity against myofibrillar and sarcoplasmic proteins (17–20).

Molds can grow and develop on the outer surface of the ham due to the humidity and temperature conditions in the curing chambers when no precautions are taken. The most common mold is *Penicillium* (21), but some yeasts, mainly *Candida zeylanoides* in early stages and *Debaryomices hansenii*, also may grow (22). The isolated molds (around 75%) have shown good antimicrobial activity against *Staphylococcus aureus* and are able to inhibit its growth (21).

V. PHYSICAL AND CHEMICAL CHANGES DURING THE PROCESS

Main chemical changes are the result of changes in composition due to water loss and salt penetration. The diffusion of water through the ham and its evaporation when reaching the surface is a slow and difficult process. Both rates must be equilibrated to achieve adequate drying and, in this sense, it is very important to have the water sorption isotherms to predict the required time for drying. Diffusion of salt is also very slow and is affected by many variables such as temperature, size of the ham, pH, amount of moisture, and intramuscular fat. It takes around 4 months to get full salt equalization through the entire piece, although the salt profile may change a little depending on the particular moisture content in each muscle. pH increases from initial values of around 5.6–5.8 to values near 6.4 toward the end of the process. PSE hams have a pH evolution similar to normal ones (8,23). This evolution constitutes a narrow range where all the enzymes and chemical reactions operate (24). However, even slight variation in pH might affect the action of muscle enzymes; for example, more intense proteolysis has been reported in low-pH hams (25).

VI. BIOCHEMICAL CHANGES DURING THE PROCESS

Many biochemical changes have been reported during the processing of dry-cured ham, most of them bring a consequence of enzymatic reactions. Some of these changes are restricted to the beginning of the process; such is the case with nucleotide breakdown reactions or the glycolysis-related enzymes and subsequent generation of lactic acid. Proteolysis and lipolysis constitute two of the most important enzymatic phenomena, responsible for the generation of compounds with direct influence on taste and aroma.

A. Proteolysis

Proteolysis consists of the progressive degradation and breakdown of major meat proteins (sarcoplasmic and myofibrillar proteins) and the subsequent generation of peptides and free amino acids. The result is a weakening of the myofibrillar network and generation of taste compounds; but its extent depends on many factors. One of the most important is the activity of endogenous muscle enzymes, which depends on the original crossbreeds (7,26) and the age of the pigs (9,10). Main muscle enzymes involved in these phenomena and their main properties are listed in Table 2. These enzymes show a great stability in long dry-curing processes like hams (15,27). Other important factors are related to the processing technology: for instance, the temperature and time of ripening will determine the major or minor action of the enzymes, and the amount of added salt, which is a known inhibitor of cathepsins and other proteases, will also regulate the enzyme action (28–31). Excessive softness in ham has been correlated with high cathepsin B activity and low salt content (32,33).

Great amounts of small peptides, in the range of 2700 to 4500 Da or even below 2700 Da, are generated during the process (34–36) although this generation may be depressed by the level of salt that inhibits muscle peptidases (37). Some of these peptides give characteristic tastes (34). Recently, several tri- and dipeptides have been isolated and sequenced (38). Final generation of free amino acids by endogenous muscle aminopeptidases is very important, reaching impressive amounts as high as several hundreds of milligrams per 100 g of ham (39–42).

B. Lipolysis

Lipolysis consists of the breakdown of triacylglycerols by lipases and phospholipids by phospholipases, resulting in the generation of free fatty acids. These fatty acids may contribute directly to taste and indirectly to the generation of aroma compounds through further oxidation reactions. Main lipolytic enzymes located in muscle and adipose tissue and involved in these phenomena are listed in Table 3. These enzymes show good stability through the full process (52–54). Although their activity also depends on pH, salt concetration, and water activity, the conditions found in the hams favor their action (55). The generation rate of free fatty acids in the muscle, especially oleic, linoleic, estearic, and palmitic acids, increases for up to 10 months during the process. Most of these fatty acids proceed from phospholipids degradation (3). After this time, a reverse trend is observed due to further oxidative reactions (53,56). In the case of adipose tissue, the rate of generation, especially of oleic, palmitic, limoleic, stearic, palmitoleic, and myristic acids, is also high up to 6 months (54). In the same way, a decrease in 14% of the triacylglycerols is observed (57).

C. Oxidation

The generated mono- and polyunsaturated fatty acids are susceptible to further oxidative reactions, giving rise to volatile compounds. The beginning of lipid oxidation is correlated to an adequate flavor development (58). In contrast, an excess of oxidation may lead to off-flavors. In fact, the generation of the characteristic aroma of dry-cured meat products correlates with the beginning of lipid oxidation. Free radical formation is catalyzed by muscle oxidative enzymes, like peroxidases and cyclooxygenases, external light, heating,

Table 2 Proteolytic Muscle Enzymes and Main Properties

	EC number	Main action	Main substrate	Product	Opt. pH	Opt. Temp (°C)	Stability	Effect of salt	References
Cathepsin B	3.4.22.1.	Endo-protease	Proteins	Polypeptides	6.0	37	Years	Inhibition	29
Cathepsin L	3.4.22.15.	Endo-protease	Proteins	Polypeptides	6.0	30	Years	Inhibition	27
Cathepsin D	3.4.23.5.	Endo-protease	Proteins	Polypeptides	4.0	40	Months	Inhibition	28
Cathepsin H	3.4.22.16.	Endo-protease	Proteins	Amino acids	6.8	37	Months	Inhibition	29
Calpain I	3.4.22.17.	Endo-protease	Proteins	Polypeptides	7.5	25	Days	Activation	43
Calpain II	3.4.22.17.	Amino/endo	Proteins	Polypeptides	7.5	25	Days	Activation	43
TPP I	3.4.14.9.	Exo-protease	Polypeptides	Tripeptides	4.0	37	Months	Inhibition	3
TPP II	3.4.14.10.	Exo-protease	Polypeptides	Tripeptides	7.0	30	Months	Inhibition	3
DPP I	3.4.14.1.	Exo-protease	Polypeptides	Dipeptides	5.5	50	Months	No effect	44
DPP II	3.4.14.2.	Exo-protease	Polypeptides	Dipeptides	5.5	65	Months	Inhibition	45
DPP III	3.4.14.4.	Exo-protease	Polypeptides	Dipeptides	8.0	45	Months	Inhibition	46
DPP IV	3.4.14.5.	Exo-protease	Polypeptides	Dipeptides	8.0	45	Months	Inhibition	47
Methyonyl	3.4.11.18.	Aminopeptidase	Peptides	Amino acids	7.5	40	Years	Inhibition	48
Alanyl	3.4.11.14.	Aminopeptidase	Peptides	Amino acids	6.5	37	Years	Inhibition	49
Leucyl	3.4.11.1.	Aminopeptidase	Peptides	Amino acids	9.0	45	Years	No effect	50
Pyroglutamyl	3.4.19.3.	Aminopeptidase	Peptides	Amino acids	8.5	37	Weeks	Inhibition	31
Arginyl	3.4.11.6.	Aminopeptidase	Peptides	Amino acids	6.5	37	Months	Activation	51

Table 3 Lipolytic Muscle and Adipose Tissue Enzymes and Main Properties

Enzyme	Main action	Main substrate	Opt. pH	Opt. temp. (°C)	Stability	Effect of salt	References
Muscle							
Acid lipase	Lipase	Triacylglycerols	5.0	37	Months	Activation	52,53
Neutral lipase	Lipase	Triacylglycerols	7.5	45	Years	Inhibition	52,53
Phospholipase A	Phospholipase	Phospholipids	5.0	37	Months	Activation	3
Acid esterase	Esterase	Triacylglycerols	5.0	30	Years	Inhibition	52,53
Neutral esterase	Esterase	Triacylglycerols	7.5	20	Years	Inhibition	52,53
Adipose tissue							
Hormone-sensitive lipase	Lipase	Triacylglycerols	7.0	37	Months	Activation	52,54
Monoacylglycerol lipase	Lipase	Monoacylglycerols	7.0	37	Months	Activation	1
Lipoprotein lipase	Lipase	Lipoproteins	8.5	37	Months	Inhibition	1
Acid esterase	Esterase	Triacylglycerols	5.0	60	Years	Inhibition	52,54
Neutral esterase	Esterase	Triacylglycerols	7.5	45	Years	Inhibition	52,54

and the presence of moisture and/or metallic cations. The next step in oxidation is the formation of peroxide radicals (propagation), by reaction of free radicals with oxygen. The formed hydroxyperoxides (primary oxidation products) are flavorless but very reactive, giving secondary oxidation products that contribute to flavor (59). Oxidation is finished when free radicals react with each other. Main products from lipid oxidation (60–62) are aliphatic hydrocarbons (poor contribution to flavor), alcohols (high odor threshold), aldehydes (low odor threshold), and ketones. The last two groups are related to the aroma of dry-cured ham in French-type hams (63) and Spanish hams (64,65). Alcohols may interact with free carboxylic fatty acids, giving esters, especially when nitrate is not used, as in Parma ham, in which esters are generated in greater amounts and are well correlated with its aged odor (66).

VII. DEVELOPMENT OF SENSORY CHARACTERISTICS

A. Color

The color of dry-cured ham mainly depends on the concentration of its natural pigment myoglobin, which depends on the type of muscle and the age of the animal (67,68). So, myoglobin concentration is higher in muscles with oxidative pattern and in older animals. The typical bright-red color is due to nitrosomyoglobin, a compound formed after reaction of nitric oxide with myoglobin. About 10–40% of total myoglobin is transformed into nitrosomyoglobin (69). Those hams without added nitrate present a pinky-red color. Some surface colors on smoked hams may result from the pyrolytic decomposition of wood.

B. Texture

The texture of dry-cured hams depends on several factors, such as the extent of drying (loss of moisture), the extent of proteolysis (degree of myofibrillar protein breakdown), and the content of connective tissue (70). In fact, major structural proteins such as titin, nebulin, and troponin T are fully degraded whereas myosin heavy chain and α-actinin are partly proteolyzed (27,71). Some small fragments (150, 85, 40 and 14.4 Kda) appear as a consequence of proteolysis (1,24). The content of intramuscular fat also exerts a positive influence on some texture and appearance traits (72).

C. Flavor

1. Generation of Taste Compounds

Glutamic and aspartic acids impart an acid taste and sodium salts give ham a salty taste. Bitter taste is mainly associated with aromatic amino acids such as phenylalanine, tryptophan, and tyrosine; sweet taste with alanine, serine, proline, glycine, and hydroxyproline (34). The generation of all these free amino acids is extremely important in dry-cured ham (36,39,41) and is somehow affected by levels of salt (41,73). For instance, lysine and tyrosine are well correlated to an improvement in the aged taste of Parma ham (66), although in other cases, such as in French-type dry-cured ham, only a small effect on flavor development has been reported (63). An excess of proteolysis (proteolysis index higher than 29–30%) is undesirable because it may give a bitter or metallic aftertaste (66,74).

Specific tastes for dry-cured ham have been found after fractionation by gel filtration chromatography in several fractions with low molecular mass, below 2700 Da, accompanied by some nucleotides and a few compounds from protein-lipid interaction (34).

Table 4 Main Groups of Volatile Compounds Generated During the Processing of Dry-Cured Ham

Groups volatile compounds	Main origin	General characteristic aromas
Aliphatic hydrocarbons	Autooxidation of lipids	Alkane, crackers
Aldehydes	Oxidation of free fatty acids	Green, pungent, fatty
Branched aldehydes	Strecker degradation of amino acids	Roasted cocoa, cheesy-green
Alcohols	Oxidative decomposition of lipids	Medicinal, onion, green, alcoholic
Ketones	β-keto acid decarboxylation or fatty acid β-oxidation	Buttery, floral, fruity
Esters	Interaction of free carboxylic acids and alcohols	Fruity
Nitrogen compounds	Maillard reaction of amino acids with carbohydrates	Meaty, nutty, toasted nuts
Sulfur compounds	Sulfur-containing amino acids	Dirty socks
Furans	Sulfur-containing amino acids with carbohydrates	Hamlike, fishy

Source: Ref. 3.

Some of these tasty peptides, mainly di- and tripeptides, have been successfully purified and sequenced (37). Free amino acids may also serve as a source of volatile compounds during further ripening (75) or when the ham is heated, like the country-style ham (76).

2. Generation of Aroma Compounds

Aroma development in dry-cured ham is a very complex process involving numerous reactions such as chemical or enzymatic oxidation of unsaturated fatty acids and further interactions with proteins, peptides, and free amino acids (61,77). In fact, more than 200 volatile compounds have been reported in dry-cured hams (60, 64,78–80) as summarized in Table 4. Some volatile compounds, such as 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal, arise from Strecker degradation of the amino acids valine, isoleucine, and leucine, respectively. Some pyrazines formed through Maillard reactions between sugars and free amino acids, although in low amounts, also impart some characteristic aromas like nutty, green, earthy, and so forth. Final flavor depends on the mixture of characteristic aromas and odor thresholds for each compound, although, in general, ketones, esters, aromatic hydrocarbons, and pyrazines are correlated with pleasant aroma of ham (81). Some correlations have been found between some volatile compounds and specific characteristics of the process. For instance, the correlation of aged flavor of Parma ham with short-chain methyl-branched aldehydes, esters, and alcohols (66,82); hexanal, 3-methyl butanal, and dimethyl disulfide with short drying processes (64); or methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters, and dimethyl trisulfide with nutty, cheesy, and salty descriptors (83).

VIII. ACCELERATED PROCESSING OF DRY-CURED HAMS

Many attempts have been made to accelerate the process, especially in country-style hams. Most of them try to accelerate the penetration and diffusion of salt into the hams, such as

by boning and skinning of hams (84–86), mechanical tenderization through blade penetration prior to dry cure (87), tumbling in a revolving drum with baffles (88), or the direct use of nitric oxide instead of nitrate or nitrite (89). In other cases, more intense biochemical changes through papain injection (90), microbial inoculation (91), or membrane disruption by prefreezing and thawing of hams (92,93). Future developments will include the addition of microorganisms with a determined enzyme profile or just a purified enzyme, microencapsulated in lyposomes or rubbed onto the outer surface of the hams, with specific roles to reinforce the action of the endogenous enzymes during the process.

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22
Semidry Fermented Sausages

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I. BACKGROUND DEFINITIONS AND CLASSIFICATION

Semidry sausages are almost always considered at the same time as the dry sausages, probably because these two types of sausage products are most often fermented and consequently share many production similarities. However, there are significant differences between these two products in finished form. The semidry sausages are softer in texture and characterized by a markedly different flavor profile than dry sausages. Semidry sausages are typically products that are fermented and cooked but are not usually dried beyond the moisture lost during fermentation and heating. Because finished semidry sausages typically retain at least 80–85% of the moisture content of the initial formulation, these products remain softer in texture than fully dried sausages. Further, because the higher moisture content makes these products more susceptible to spoilage, they are usually fermented to a low pH, resulting in a very tart, tangy flavor.

Undoubtedly, both semidry and dry sausages developed simultaneously out of the need to preserve a very perishable food. History indicates that the ancient Egyptians were among the first to record the preservation of meat by salting and sundrying (1). Adding salt would have resulted in chance fermentations at warm temperatures. Once the preservation and culinary advantages of fermented products were realized, observant individuals began to deliberately encourage fermentation processes. China is believed to be another originator of fermented sausage products, some 2500 years ago (2); there large amounts of sugar were included for additional preservation effects. By the 1700s, fermented sausages had developed into several different versions such as the dry and semidry varieties. These products became relatively common around the Mediterranean and subsequently in Germany, Hungary, and upper Europe.

The traditional classification of fermented sausage types has been derived from historical origins, with "Italian types" most often meaning dry sausage without smoke and with a relatively mild acid flavor or tang. "Germanic type," in contrast, most often describes semidry products that have been smoked and that have a greater tartness or tang from the fermentation. Semidry sausages have also been produced in a number of varieties. In addition to the Germanic type, found in Germany, The Netherlands, Scandinavia, and the United States, there are some French and Spanish products that are characterized by a short drying period in which external mold growth is encouraged (3). An unique semidry

Figure 1 Semidry summer sausage and thuringer available as several varieties, including garlic, lite, all-beef, and regular (beef/pork) formulations. (Photo courtesy of Klement's Sausage Co., Milwaukee, WI, USA.)

sausage is the Lebanon Bologna produced in the eastern United States. These products are all-beef, very heavily smoked, and fermented to a very low pH (about 4.5). In addition, high carbohydrate levels are included in the formulation of Lebanon Bologna to result in a significant amount of remaining sugar following the fermentation. The result is a sweet, tangy, smoked flavor that is unique to this product.

Semidry sausage is found in several different forms, but it is most often characterized by fermentation to relatively low pH, addition of smoke, heating for pasteurization, and little or no drying. Semidry sausage usually has a pH of 5.3 or less and a water activity of 0.86 or higher. Figure 1 shows a variety of summer sausage and thuringer available as garlic, "lite," and all-beef formulations in addition to conventional beef/pork formulations.

II. CHARACTERISTICS AND REQUIREMENTS

Semidry sausages may be defined on the basis of available water (A_w) or moisture-to-protein (M:P) ratios. The product standards based on A_w are more common in Europe; the US uses M:P ratios. Generally speaking, semidry sausages in the United States are defined by M:P ratios of 1.9:1 or more and may be as high as 3.7:1 (4). Moisture-to-protein ratios of some typical products as required by the United States Department of Agriculture (USDA) for US products, both dry and semidry, are shown in Table 1. Most of the semidry sausage products require refrigeration unless a specific combination of pH and moisture-to-protein ratio is achieved. This combination is pH 5.0 or less and a M:P ratio of 3.1:1 or less. If these two conditions are met, the product is considered shelf-stable and may be stored at room temperature.

Table 1 Examples of Moisture:Protein Ratios Required by the U.S. Department of Agriculture for Some Semidry and Dry Sausages

Product	Ratio
Semidry sausage (with lactic acid culture)	
Thuringer	3.7:1
Summer sausage	3.7:1
Lebanon bologna	3.7:1
Shelf-stable semidry sausage	
Summer sausage with pH 5.0 or less	3.1:1
Chorizo	3.1:1
Genoa salami	2.3:1
Beef salami	2.3:1
Sicilian-style salami	2.3:1
Dry sausage	
Dry salami	1.9:1
Pepperoni	1.6:1
Jerky	0.75:1

The general objective of achieving a reduced pH by fermentation and limited drying necessary for semidry sausages can be approached in two ways. Semidry sausages in North America are most often fermented rapidly (12 hr or less) at high temperature (32–46°C) and to a relatively low pH (well below 5.0). In Europe and many parts of the world, fermentation tends to be slow (24 hr or more) at lower temperatures and to somewhat higher final pH. These differences in fermentation speed and final pH result in significantly different product flavor (5).

While the flavor, and sometimes texture, of semidry sausages can vary considerably, and can be highly acceptable in all cases, all of these products must meet a rather narrow range of conditions to assure that shelf life and safety are achieved. The reduced pH of fermented sausage is, by itself, a significant means of extending the shelf life of products and, when combined with low storage temperatures of 2°C or less, is a very effective means of preservation. However, as noted earlier, semidry sausages in the United States may also be considered shelf-stable without refrigeration if the pH is 5.0 or less and the M:P ratio is 3.1:1 or less. In addition, shelf-stable products have been defined by others in Europe as having, pH of 5.2 or less with water activity of 0.95 or less (6). Products that meet either of these requirements are very unlikely to spoil from bacterial growth, and shelf life is usually limited only by chemical or physical spoilage.

Safety from the hazards of pathogenic microorganisms is a separate issue from shelf life and one that is of critical concern to both meat processors and consumers. For semidry sausages, the relatively high temperatures often used for rapid fermentation can also accentuate the growth of pathogenic bacteria, particularly if acid production is slower than expected. Several pathogenic microorganisms are recognized as probable contaminants of raw meat under normal conditions. However, many of these are very unlikely to grow in the presence of salt, nitrite, and a dominant lactic acid culture. *Salmonella* spp., for example, are inhibited by salt and by growth of the starter culture; *Clostridium botulinum* and *Clostridium perfringens* are very effectively controlled by the presence of nitrite. These pathogens have very rarely been associated with food-borne illnesses originating from semidry sausages. However, pathogens that are salt- and nitrite- tolerant have the potential to survive and

grow in semidry sausages. Two pathogens that have been long recognized as significant risks in fermented sausage are *Trichinae spiralis* and *Staphylococcus aureus*.

T. spiralis, if present, will survive fermentation but is easily killed by heat. Practically speaking, this pathogen is a risk only in unheated products, and because of recent requirements to inactivate other "new" pathogens such as *Escherichia coli* O157:H7, heat treatments for semidry sausages have become common and are typically more than adequate to inactivate *T. spiralis*. In addition, modern swine production practices have resulted in near-elimination of *T. spiralis* from pork. Consequently, concern for *T. spiralis* in semidry sausages is no longer a major issue, but it should be noted that regulations still apply for trichinae control in pork products. These regulations specify that trichinae-free pork ("certified pork" in the United States) must be used for products that do not receive sufficient heating to kill the organism. Heating products to an internal temperature of 62.2°C is sufficient to inactivate this organism.

Staphylococcus aureus has been one of the "trademark" pathogens associated with fermented sausage, and care is required to prevent food-borne illness outbreaks from this organism. *Staphylococcus* is a relatively common contaminant in raw meat and one that is very salt- and nitrite-tolerant (1). This organism can produce a heat-stable enterotoxin (7) that survives heat processes even when the organism does not. It is not unusual to have a scenario in which a product has caused illnesses from staphylococcal toxin but no organisms can be recovered because they were killed by heating while the toxin remained viable. Fortunately, *Staphylococcus aureus* is not very tolerant of acid conditions, and a critical control point for control of this organism in fermented sausage is achieving pH 5.3 before the organism can produce toxin. Because staphylococcal toxin can be produced in fermented sausage when temperature exceeds 15.6°C, use of "degree-hour" limits are used to prevent toxin production. These limits are defined as the time in hours multiplied by the temperature in degrees Fahrenheit in excess of 60°F (15.6°C) (1). For example, a product that reached pH 5.3 in 20 hr at 100°F would have experienced (100°F–60°F) × 20 hr = 800 degree-hours. Table 2 shows the maximum degree-hours allowed to reach pH 5.3 to assure control of *Staphylococcus aureus* toxin production. For semidry sausages fermented at 90–100°F (32.2–37.8°C) or higher, the degree-hours required is 1000; for fermentation temperatures of 100–110°F (37.8–43.0°C), the requirement is 900. Any product that reaches pH 5.3 in 18 hr or less would be considered safe from staphylococcal enterotoxin production during fermentation. The degree-hour concept assumes a constant temperature process during fermentation.

Since the mid-1990s, when *E. coli* O157:H7 was found in fermented sausage and determined to be the cause of food-borne illness outbreaks, additional requirements have

Table 2 Degree-Hours to Achieve Control of *Staphylococcus aureus* Toxin Production During Sausage Fermentation

Maximum degree/hours[a] to achieve pH 5.3	Temperature	Maximum hours to meet degree/hour limit
1200	80°F (23.9°C)	60
1000	90°F (32.2°C)	33
1000	100°F (37.8°C)	25
900	105°F (40.4°C)	20
900	110°F (43.0°C)	18

[a] Degrees Fahrenheit in excess of 60°F multiplied by the time in hours, assuming temperature is constant.

been implemented to assure safety of semidry (and dry) sausage products. Organisms such as *E. coli* O157:H7 and *Listeria monocytogenes* are cold-tolerant organisms that can survive fermentation. Consequently, additional requirements in the United States have been imposed by the USDA to assure safety in fermented sausage products. Processors are required to show that the process being used is effective for destruction of *E. coli* O157:H7. *Validation* of the process must demonstrate at least a 5-log (100,000) inactivation of the organism using USDA protocols. The most common means of achieving this is by heating. For example, USDA regulations include time-temperature combinations for heated products that will achieve *E. coli* inactivation such as 145°F (62.8°C) for 4 min (8). Processors also have the option to hold and test finished products for *E. coli* O157:H7 contamination to show that the product is safe. A third alternative for assuring safety is to use a hazard analysis–critical control points (HACCP) program that includes testing of the raw product mixture and a minimum 2-log (100) kill. All of these process options for controlling *E. coli* O157:H7 must also be shown to be adequate to control trichina and salmonella. One concern for the heating process is that it can change the textural properties of finished products. Irradiation provides a viable alternative to the heat process for inactivation of *E. coli* O157:H7 and other pathogens in meat to be used for fermented sausage (9). A relatively low irradiation dose of 1.25 kGy is sufficient to provide a 5-log kill of *E. coli* O157:H7 (10). An advantage to irradiation is that meat is unaltered and retains raw characteristics in terms of color and texture. Semidry (and dry) sausage can be manufactured from irradiation-treated meat ingredients without the heating requirement for *E. coli* control. The resulting product characteristics, especially texture, more closely resemble that of traditional products (11). While irradiation has been approved in the United States for most fresh meat applications, approval of irradiation for finished processed products has not been finalized. Irradiation is fully capable of increasing shelf life and safety of finished semidry sausage products, but questions relating to product quality changes, requirements for packaging materials, and appropriate doses have precluded regulatory approval thus far.

III. MEAT INGREDIENTS

Because of the warm, humid conditions normally utilized for fermentation of semidry sausages, meat ingredients must be of very high quality. Fermentation encourages microbial growth and accelerates chemical reactions, both of which have potential to affect final product quality and may even result in spoilage. Fresh meat with no microbiological or chemical age is essential for manufacturing fermented sausage. If initial microbiological counts on meat are high, undesirable growth may occur during fermentation even if a starter culture is added. If chemical changes have begun to occur, most notably lipid oxidation, they may then accelerate under the conditions used for fermentation and result in significant off-flavors.

Problems in fermented sausage that may result from low-quality meat ingredients can also include "greening," a color change from peroxides produced by undesirable bacteria. The peroxides typically are produced during fermentation and may cause greenish colors from the meat pigment when the pigment is exposed to air. Other problems that can develop from undesirable bacterial growth during fermentation include a mushy texture, resulting from proteolysis by some organisms. Production of carbon dioxide gas during fermentation is highly undesirable and may occur if high numbers of heterofermentative microorganisms are present. The result of gas production is gas bubbles in the product or even breakage of casings if gas production is extreme. Product pH may be altered if micrococci are present in the early stages of fermentation. Some conversion of carbohydrates may occur without

formation of lactic acid and this could result in a higher final pH in the product than expected. Fresh meats should also be well trimmed of glands, blood clots, and other nonmuscle materials. Glandular materials, in particular, can be a source of spoilage microorganisms, proteolytic enzymes, and pathogens such as *Staphylococcus aureus*.

Meat trimming also becomes important for the removal of connective tissue originating from sinews, tendons, and similar structures in skeletal muscle. Most semidry sausages are not heated to the extent that hydrolysis and tenderization of the collagen in connective tissue occurs. Further, the semidry sausage products are typically ground or chopped to a relatively coarse texture. This means that any connective tissue in the product will remain tough and will be present in large enough particles to be detected during consumption. Excessive collagen may also appear as unattractive "fish eyes" when a product is sliced. Removal of connective tissue is an important quality control point for semidry sausages, and many processors employ desinewing machines for the meat ingredients that are designated for semidry sausage formulations.

The color of both lean and fat of the meat materials will affect semidry sausage quality. Yellow fat, for example, is not acceptable for the appearance of semidry sausage. Generally speaking, more intensely pigmented (redder) lean is preferable to provide a greater color contrast (red lean/white fat) in the finished product. This is one reason why pork shoulder meat is preferable to the paler ham and loin trimmings as a meat ingredient for semidry sausage (12).

Chemical quality, particularly of the fat, is also a concern for meat ingredients. Fat begins to oxidize almost immediately after slaughter, but the oxidative reactions occur very slowly at first. As time goes on, the oxidation reactions that produce rancid flavors accelerate and continue to do so in a logarithmic fashion. Because of this, it is extremely important that meat ingredients have minimal oxidation history prior to use for semidry sausage. This is one area where frozen meat, if used, should be carefully chosen for these products. Frozen meat is often held for variable periods of time in frozen storage and slow oxidation occurs even though microbial growth may not. An "old" frozen meat ingredient may trigger rapid rancidity development if the chemical quality is poor coming out of the freezer.

The initial pH of the raw meat ingredients may also be an important consideration for fermented sausage. Generally, a lower initial pH will facilitate the pH change desired during fermentation and will permit shorter fermentation time. For this reason, dark, firm, and dry (DFD) pork and dark-cutting beef are not ideal meat ingredients because these meat sources will have a high initial pH. In the case of pork, it is not uncommon to encounter pale, soft, and exudative (PSE) pork with a lower-than-usual initial pH. This meat ingredient will ferment more quickly and releases moisture readily but will also result in a crumbly, mushy texture in fermented sausage because meat proteins have been damaged. The pale color inherent to PSE pork can also result in uneven color in the finished product, depending on the proportions used. Therefore, PSE pork, even though it sometimes has a low initial pH, is very undesirable as a meat ingredient for semidry sausage.

Meat ingredients may also affect fermentation and fermentation rates in other ways. For example, a lean product formulation will contain higher moisture content than a high-fat formulation and with more moisture, the fermentation may develop more quickly. There has also been a suggestion that glycogen (carbohydrate in muscle) content of lean meat may contribute to total acidity. However, residual glycogen in lean muscle is typically quite low and generally would not be expected to contribute significantly to fermentation.

Meat sources derived from different muscles and from different animal species may demonstrate significantly different buffering capacity. Meat with a greater buffering capacity will absorb more acid with less pH change during fermentation and would require

more acid production to reach a target pH. In general, beef is viewed as having a greater buffering capacity than pork and, in addition, is often at a somewhat higher initial pH. Consequently, it typically takes longer to ferment an all-beef product than a pork-beef mixture. An additional hypothesis suggested for the different fermentation rates between beef and pork is that pork contains a much greater concentration of thiamine, which could serve as a microbial growth stimulant and facilitate faster growth rates by the culture (1).

IV. NONMEAT INGREDIENTS

The fundamental nonmeat ingredients essential to semidry sausages include carbohydrates/sugars, salt, curing agents, and spices. In addition, starter cultures or acidulants are essential in most cases unless a traditional fermentation or backslopping-type inoculation is being used.

A variety of carbohydrates will provide the necessary substrate for production of lactic acid during fermentation, but the simple sugars, such as dextrose, are preferred. Dextrose is most commonly used because it is very readily utilized by all lactic acid bacteria. Final product pH will be directly related to the amount of dextrose in the product formulation, up to about 0.6%–0.7%. Most semidry sausages are formulated with 0.7%–1% dextrose. Greater amounts of dextrose (1.0% or more) will not greatly affect final product pH because the culture cannot grow in the presence of excess acid. Sucrose will also provide acid production and pH change very similar to dextrose and may be used in product formulation. A number of other carbohydrates such as maltose and lactose have been studied as fermentation substrates and will result in lactic acid production. However, most of these carbohydrates result in somewhat slower fermentation and higher final pH compared with dextrose (13). Figure 2 shows the pH differences achieved by five different carbohydrate sources during a meat fermentation. Complex carbohydrates such as corn syrups can be fermented, but the rate and extent of the fermentation depends on the amounts of simple sugars present in the mixture (usually expressed as dextrose equivalent)(13). Excess carbohydrates may be used in some cases where sweetness is desired for part of the final flavor profile along with acid tartness. The Lebanon Bologna products produced in the eastern United States are one such example where as much as 2% dextrose may be included. Sweetness from residual sugar tends to moderate the tartness of lactic acid and provides for a less astringent flavor.

Salt is an absolutely essential nonmeat ingredient for nearly all processed meat products as well as semidry sausages. For example, salt immediately inhibits the gram-negative organisms typically found in raw meat and allows the gram-positive lactic acid bacteria to predominate. Thus, salt is essential to achieving fermentation. Most sausage products will contain 2.0–3.5% salt, with the level chosen dependent on the flavor and texture desired. Although the lactic acid bacteria are salt-tolerant, it has been demonstrated that salt levels over 3.0% are likely to slow down fermentation, especially at low temperatures of 75–80°F (~26°C). One concern for this effect of salt is that *Staphylococcus aureus* is somewhat more salt-resistant than the lactic acid bacteria. A high salt concentration of more than 3.5% could favor the growth of this pathogen during fermentation (1).

Curing agents used in semidry sausages are primarily sodium nitrite, though sodium nitrate may be used as well. Because nitrite is the active curing ingredient that results in the typical cured meat characteristics, nitrate is generally unnecessary, except in some cases such as dried meat products. In dried products, nitrate can provide a reservoir of nitrite to maintain color and other cured meat characteristics over extended periods of time. Because

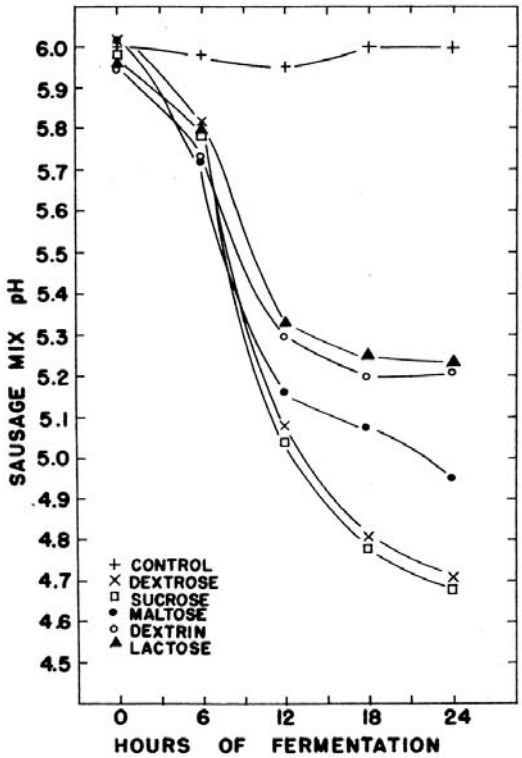

Figure 2 Change in pH during sausage fermentation using 1% of different carbohydrates. (Control has no carbohydrate.) (From Ref. 13. Courtesy of J. Acton, Clemson University, Clemson, SC, and the Institute of Food Technologists, Chicago, IL.)

nitrite is a good microbial inhibitor, it can affect the starter culture. This means that care should be taken to prevent the culture from directly contacting nitrite during formulation and mixing of product batches. It is recommended that nitrite be added to meat and mixed well prior to addition of culture to prevent localized areas of high nitrite concentrations when the culture is added.

Spices are used for flavoring of sausage products but can also have a direct effect on fermentation rates. Some natural spices have been shown to stimulate acid production because they contain significant amounts of manganese (14). Manganese provides an essential growth element for cultures and facilitates faster acid production. Spices that have been found to stimulate acid production include pepper, mustard, garlic, allspice, nutmeg, ginger, mace and cinnamon (1). Many of these spices are common in semidry sausage formulations. Starter culture growth stimulated by addition of manganese has been shown to have improved inhibitory effects on pathogens including *L. monocytogenes* and *S. aureus* (15). On the other hand, there is also evidence that natural spices can also be inhibitory to microbial growth. There have been reports that spices, especially spice extracts, resulted in reduced fermentation rates (16). However, this does not appear to be a major concern for fermented sausage. Most of the current studies on spices as inhibitors are directed at the control of pathogens in unfermented products (17).

Starter cultures have become a common nonmeat ingredient for semidry sausages; a large majority of fermented sausages are now produced using a commercial lactic acid

starter culture. Adding a commercial culture assures meat processors of a pure strain of desirable organisms and provides sufficient number of these organisms to overwhelm any undesirable organisms that may be present. Cultures are generally available in frozen or freeze-dried form but must be handled appropriately to retain maximum viability. For example, thawing the culture in hot water or dispersing the culture in chlorinated water may adversely affect activity and subsequent fermentation rates. There are several specific cultures available, yet it is the first commercial starter, developed in 1957, that remains most common in semidry sausage products. This organism was originally identified as *Pediococcus cerevisiae* but later (1974) reclassified as *Pediococcus acidilactici* (18). Pediococci strains (including *P. pentosaceus*) have been the most popular for semidry products because fermentation by these organisms is rapid at relatively high temperatures (optimum is 40°C) and many different carbohydrates can be utilized. These organisms produce a sharp, tangy flavor which is preferred in most semidry sausages.

Other relatively common organisms found in starter cultures include lactobacilli. Although most lactobacilli are used for dry sausage, *Lactobacillus plantarum* is a strain that is often used for semidry products. This organism has an optimal growth temperature of 30–35°C and is often used as a mixed starter culture in combination with *P. acidilactici*. These organisms work well together, but mixed cultures are not always fully compatible, and combining two fast-fermenting organisms will not necessarily result in the most active culture (19). Other commercially available cultures around the world include several other lactobacilli and some nonpathogenic staphylococci. Most of these, however, are used for dry sausage products that are fermented at relatively low temperatures. Micrococci are also supplied in some mixed starter cultures for meat fermentations. These organisms are not lactic acid producers but are included to reduce nitrate to nitrite. This is a viable function for the culture in dry sausage products but is normally unnecessary in semidry products where nitrite-only cures are more commonly used. Recent research has suggested that some probiotic lactic acid bacteria, common to the human intestinal tract, could be effectively utilized for meat fermentations (20). *Lactobacillus gasseri* was most effective among several strains studied.

In addition to the "biological acidulation" achieved by starter cultures, a number of meat processors have begun utilizing "chemical acidulation" with encapsulated acids. The acids used are in the form of small droplets covered with a lipid coating, which is composed of hydrogenated cottonseed and soybean oil. The coating melts when heated to 57°C and slowly releases the acid into the meat mixture. The release of acid is slow enough to produce textural effects similar to a biological fermentation, yet fast enough to save a considerable amount of time. Acids available in encapsulated form include lactic acid, which is the same compound as that produced by starter culture, citric acid, and glucono delta lactone (GDL). The encapsulated lactic acid product is about 30% acid by weight; consequently, addition of 3.0% is necessary to release about 0.9% acid into the product and achieve a pH of about 4.9. Encapsulated citric acid is about 70% acid by weight, so addition of 0.7% will release about 0.5% acid in the product with a resultant pH of about 4.8. Encapsulated GDL is also about 70% GDL by weight but in this case a 0.7% addition will produce a pH of about 5.3. Addition of 1.5% of the encapsulated GDL is necessary to achieve a pH of 4.9.

A recent development for nonmeat ingredients in semidry sausages has been the addition of nonmeat proteins. Nonmeat proteins such as soy may provide an opportunity to reduce formulation costs and/or reduce fat content while retaining expected product characteristics. In the United States, addition of up to 2% soy isolate allows declaration of soy protein in the ingredients list of the product without major label changes. The proteins are available in structural form to provide meatlike texture and may include coloring to be compatible with finished product color (21). Unstructured soy protein may also be used in

cases where a soy protein/water gel provides texture to replace fat. While the structured protein resembles meat in texture, the gelled protein has a texture more similar to fat.

V. FORMULATION/MIXING/STUFFING

One of the most important considerations for semidry sausages during formulation, mixing, and stuffing into casings is good temperature control of the meat ingredients. Fresh (unfrozen) ingredients must be as cold as possible, preferably $-5°C$ to $-2°C$ prior to the first grinding or chopping treatment. Frozen meat is often blended with unfrozen meat to maintain low temperatures. The primary purpose of the low temperature is to maintain clearly defined fat particles and a sharp fat-to-lean color contrast when the finished product is sliced. Good temperature control also prevents fat smearing during mixing and stuffing, an effect that is more critical for products that are dried following fermentation. Some processors will chill fat meat sources to temperature as low as $-15°C$ to $-12°C$ to produce the best possible particle definition.

Mixing time for semidry sausages should be limited to the minimum necessary to achieve good dispersion of all ingredients. Curing ingredients and spices should be added first, salt should be added toward the end of the mixing cycle, and culture should be added last after all other ingredients are well dispersed.

Many processors prefer to use choppers for semidry sausages because the cutting action of the chopper blades results in cleanly cut particles. A potential disadvantage to using a chopper is that the fat and lean particles are seldom uniform in size. Grinders provide for particle size uniformity and have an additional advantage in that bone removal systems can be utilized. The disadvantage to a grinder is the likelihood for greater fat smearing and less particle differentiation.

When mixers are used, a paddle mixer or blender is generally considered to be superior to ribbon-type mixers because of the need to prevent fat smearing. A closed mixer that can be operated under vacuum is also valuable for removal of air. Removing air during mixing has several advantages, including better appearance (no air bubbles or vacuoles), better color stability, and improved flavor stability.

A number of different casings are available for semidry sausages, including natural, collagen, or cellulose casings. These casings differ in appearance, strength, and uniformity. Natural casings provide the most traditional appearance whereas collagen and cellulose offer much better uniformity and, in the case of cellulose, strength as well. Regardless of the casing chosen, however, it is important to stuff casings to the manufacturer's recommended diameter to avoid air pockets, wrinkling, or splitting. Stuffing horns need to be correctly sized to the casing and free of dents and defects in order to minimize fat smearing during stuffing.

VI. FERMENTATION AND FINISHING

After stuffing, semidry sausages are typically hung on sticks, placed on racks, and moved to fermentation chambers. The conditions for fermentation should provide optimum temperature, humidity, and time for the chosen lactic acid–producing bacteria. These conditions may range from about $27°C$ to $43°C$ with 95–99% relative humidity, depending on the culture used. Appropriate conditions will allow rapid culture growth to overwhelm undesirable organisms, rapid production of acid to control pathogens, and sufficient produc-

tion of other metabolic products to result in expected product characteristics. Semidry sausages are typically fermented at relatively high temperature (over 35°C) to shorten fermentation time and allow greater production capacity. Fermentation in 12 hr or less is not unusual for the high temperature processes.

Although relatively high fermentation temperatures are characteristic of semidry sausages, it is important that product temperature be raised relatively slowly when the product is first being heated. Initially, cold product surfaces are likely to develop surface condensation when exposed to warm, humid air and blotchy colors may develop if the surface condensation is not well controlled. Uneven heating is another potential problem that can result from condensation because water will increase in temperature more slowly than fat. If fat is overheated, it is likely to separate, resulting in a greasy product surface or fat pockets in the product.

For semidry sausages, smoke is often applied during product heating. Because smoke is a microbial inhibitor, it is best to apply smoke after the fermentation is complete and after cured color development has also been completed. An important point to note relative to the cured color development is that cured pigment formation is maximized in products fermented below pH 5.0 (22). Acid conditions greatly facilitate the development of cured color.

The final step of the heating process for semidry sausages is cooking/finishing to reach an internal product temperature that will assure product safety. In the past, many products received relatively mild heat treatments, often finishing at 60°C internal temperature. Many of these processes are no longer considered sufficient for pathogens such as *E. coli* O157:H7. Many processors now finish semidry sausages with internal temperatures of 68.3°C or more to ensure safety. An added benefit to higher finishing temperatures may be realized in longer shelf life as well.

VII. PACKAGING

When the semidry sausage has been fermented, smoked, cooked, and chilled, it is ready for distribution to consumers. However, to prevent weight losses and product contamination, a good packaging system is necessary. Clear, high-barrier films are the most common packaging material because they provide good product viewing and, with high oxygen and water barriers, will prevent color losses, flavor deterioration and weight losses. Vacuum packaging is a very effective approach and one that will also prevent surface growth of aerobic molds on semidry sausages. Gas-flush systems are becoming increasingly popular in cases where vacuum packages result in moisture accumulation (purge) on the product surface. In these packaging systems, an inert gas, most often nitrogen, is used to fill the packages after the air and oxygen has been removed. The result is a highly stable product environment that results in very little moisture loss from the product. Gas-flush packages also minimize the product shape distortion that often results from the vacuum systems.

VIII. SUMMARY

Semidry sausages are a group of processed meat products that are unique in tradition, characteristics, and technology. These products, when properly manufactured and packaged, are among the best-preserved food products available. At the same time, semidry sausages provide uniquely desirable flavor profiles and highly pleasurable eating experiences for those who partake of these products. From development in small-localized areas,

semidry sausage has become widely popular around the world. Demand for these products will continue to increase as consumers discover the variety of eating pleasures available from this group of fermented foods.

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23
Dry Fermented Sausages

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I. INTRODUCTION

The origin of fermented meat products still remains unknown, although the history of fermented sausages goes back more than 2000 years in Mediterranean countries (1). Today's sausages are still made from the ancient recipe: comminuted meat, mixed with salt and spices, and stuffed into casings, then ripened and dried. Sausages can be surface treated—smoked or molded. Industrial development in the second half of the 19th century led to use of interior as well as exterior starter cultures to control sausage manufacturing.

Fermented meat products are found in most parts of the world, although Europe is the major producer and consumer of these products. Dry fermented sausage manufacture is a very important part of the meat industry in Europe. In the European Union, fermented sausage production amounted to 689,000 tonnes in 1988 (Table 1). In 1988, Germany (40%) and the Mediterranean countries (60%) were the main producers of fermented meat products.

II. SOUTHERN- OR MEDITERRANEAN- AND NORTHERN-TYPE SAUSAGES

The above description of dry sausage manufacturing can be used to characterize fermented sausages throughout the world, although a wide variety of products exists. National or local differences result from the recipe, seasoning, type of starter culture used, degree of communition, calibration size, fermentation, and ripening conditions. However, in Europe two types of products can be distinguished: the southern and the northern sausages (2). Typical southern sausages include Italian *salami*, Spanish *salchichon*, and French *saucisson sec* manufactured in Mediterranean countries. Northern sausages include German or Hungarian salami-type manufactured in all Nordic countries.

Southern- and northern-type sausages differ in size, the presence of beef (northern), the particle size, the use of nitrate in curing salt (southern), in fungi starters (southern), smoking (northern), as well as in fermentation temperature and duration of fermentation and ripening (Table 2).

Table 1 Production of Fresh and Fermented Sausages in Europe

	Fresh and fermented sausages[a]		Dry fermented sausages[b]
	1998	1999	1988
Northern countries			
Germany	1,202,516	1,255,976	280,000
The Netherlands	123,305	139,780	20,000
Denmark	94,553	nd	2,000
Belgium	102,497	71,970	12,000
Swede	118,311	127,208	nd
Finland	125,880	116,680	nd
Southern countries			
France	355,870	353,702	95,000[c]
Italy	45,880	56,259	141,000
Spain	414,991	371,211	130,000[d]
Portugal	20,565	17,163	nd[e]
Greece	304,723	nd	9,000
Total	2,909,091	2,509,949	689,000

nd: not determinated
[a] FICT (135); it includes cooked, to be cooked and dry sausages.
[b] Fisher and Palmer (136); only dry fermented sausages.
[c] 104,450 tonnes in 2000 (135).
[d] 166,000 tonnes in 1992 (61).
[e] 18,090 tonnes in 1998 (personal communication).

Weight loss during drying is higher than 30% in southern sausages; it is higher than 20% in northern-type sausages (3–5).

Southern technology produces sausages with higher pH values than northern sausages (Fig. 1). Several studies have reported final pH values ranging between 5.2 and 5.8 in southern sausages versus 4.8–4.9 in northern sausages (4, 6). The higher pH values are consistent with lower lactate contents: 17 mmol/100 g of dry matter (DM) in southern

Table 2 Main Production Parameters of the Two Types of Dry Fermented Sausages

Parameter	Northern sausage	Southern sausage
Diameter (mm)	90	40–60
Weight (kg)	1–3	0.4–0.8
Meat species	Pork/beef 1/1	Pork
Lean meat cuts	Miscellaneous	Mainly shoulder
Fat tissue	Pork back fat	Pork back fat
Particle size (mm)	1–2	2–6
Curing salt	Nitrite	Mainly nitrate
Bacterial starters	+	+
Fungi starters	–	+
Smoking	+	–
Fermentation	20–32°C, 2–5 days	20–24°C, 2–3 days
Ripening	15 °C, 2–3 weeks	10–15°C, 3–6 weeks

Source: Refs. 4, 6, 63, and 136.

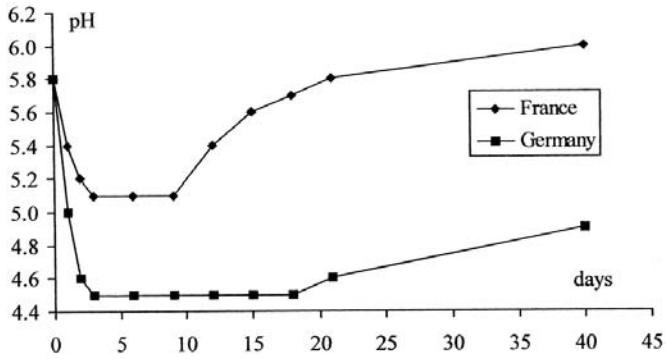

Figure 1 pH in French (southern) and German (northern) sausages.

versus 20–21 mmol/100 g DM in northern sausages (6), and 9 mg/g in French sausages versus 15 mg/g in German sausages (4). Acetate contents are also lower in southern sausages (4, 6). The pH values also depend on the ammonia content. High pH in southern products is associated with values between 10 and 14 mg N/g Nitrogen total, and low pH in northern products with smaller values (3–6 mg N/g Nitrogen total) (6).

Acidification and ammonia content largely depend on the microbial flora. Variation in the microflora during sausage manufacturing depends on several factors such as the initial contamination but also on the technology used (7). In salami manufactured according to the southern technology (addition of nitrate, low level of sugar, and fermentation at 18 °C), an increase of *Lactobacillus* and coccal gram-positive bacteria is noticed during fermentation. During ripening, the *Pseudomonas* population disappears after 7 days and the level of Enterobacteriaceae decreases. In salami manufactured according to the northern technology (addition of nitrite, high level of sugar, and fermentation at 22–24°C for 5 or 6 days), coccal gram-positive bacteria (*Staphylococcus*, *Kocuria*) in the periphery of salami and *Lactobacillus* in the whole salami grow during fermentation. Enterobacteriaceae and *Pseudomonas* populations decline very quickly and are totally inhibited within 7 days. Both processes affect the growth of populations of coccal gram-positive bacteria, which is higher in less acid products. The level of staphylococci in German salami often remains at the level of inoculation or decline during the process, whereas an increase of 1 to 1.5 log is noticed in French sausages (4). The lack of growth or inhibition of the coccal gram-positive bacteria in the northern technology can be explained by their sensitivity to acidity and anaerobiosis (8). Similarly, the inhibition of undesirable flora (Enterobacteriaceae and psychrotrophs) is noticed from the early stages of processing in nitrite-made sausages compared to nitrate-made sausages (9).

Both northern and southern processes use starter cultures for manufacturing sausages. The bacteria used as starters are inoculated at a rate of 10^6 viable germs per gram of mixture (10). Bacterial starters are generally made up of a balanced mixture of lactic acid bacteria (*Lactobacillus*, *Pediococcus*) and coccal gram-positive bacteria (*Staphylococcus*, *Kocuria*) (5). But some differences are noticed between the northern and the southern technology. In southern countries, small traditional producers rely on their own "house" flora and thus do not inoculate their sausages. In Italy, some producers use pure cultures of staphylococci to avoid acidification. In Spain, the backslopping technique (i.e., addition of some meat from a previous successful fermentation) is very common in the

production of chorizo. In northern countries, rapid acidification is searched for, so starter producers select lactic acid bacteria that acidify very quickly.

In Mediterranean countries, yeasts (*Debaryomyces*, *Candida*) and molds (*Penicillium*) are also inoculated onto the surface in order to make up the covering flora of dry sausages. In northern countries, smoking controls the growth of the external flora.

Southern and northern products are characterized by specific flavors and safety risks; therefore producers at the industrial scale are obviously interested in understanding mechanisms that control safety and flavor development in both types.

III. SAFETY RISKS IN SOUTHERN- AND NORTHERN-TYPE SAUSAGES

Raw materials (pork and/or beef lean and pork fat) are contaminated during slaughter and contamination increases during manufacturing. The contaminating flora colonizes the workshop environment and the products in a continuous symbiotic exchange. Such a flora includes microorganisms useful to fermentation and flavor formation but also spoilage and pathogenic species. The evolution of this spoilage and of the pathogenic flora and consequently product safety depend on many factors, including the manufacturing process and the use of starter cultures selected for their role in hygienic and sensory qualities of the products.

A. Pathogenic Bacteria

Pork has been described as a source of human salmonellosis and, indeed, pigs are important carriers of *Salmonella* (11,12). *Salmonella* strains disseminate and multiply during slaughtering and cutting and are commonly found on pork carcasses and cuts (13,14). *Salmonella* have been shown to survive in naturally fermented pepperoni and Lebanon Bologna (15,16). Addition of starter cultures during manufacture of pepperoni and Lebanon Bologna decreases the viability of *Salmonella*. Rapid pH drop to below 5.3 proved to be important for inhibiting *Salmonella* when products are fermented at temperatures above 18°C (17). Growing conditions of *Salmonella* are a minimal pH of 5.0 and a minimal water activity (a_w) of 0.95 (18). Thus, inhibition of *Salmonella* is mainly obtained by acidification by lactic acid bacteria in the norther technology and by drying in the southern one.

Staphylococcus aureus can be widely disseminated in meat because it is endemic in the processing environment (19). A study of pig hindquarters showed that 22.7% of the samples were contaminated by *S. aureus*, and counts from 10^3 to 10^6 cfu/cm^2 were obtained in 10% of the positive samples (20). One or more staphylococcal enterotoxin genes were identified by PCR in 17% of samples of raw pork, salted meat, and ready-for-sale uncooked smoked ham (21). In the United States, the growth of *S. aureus* and the presence of enterotoxins in fermented sausages, particularly in Genoa and Italian-type dry salami, have caused various outbreaks of food poisoning (22). Growth of *S. aureus* is inhibited at pH 5.1 and a_w 0.86 in aerobiosis, and at pH 5.7 and a_w 0.91 in anaerobiosis (18). In the norther technology, the large diameter favors anaerobiosis and pH drops very quickly below 5.0; these conditions will inhibit the growth of *S. aureus*. In the southern technology, the small diameter of the sausages favors aerobiosis, pH is often superior to 5.1, and a_w of 0.84–0.88 is only reached at the end of the drying period. None of the strains of lactic acid bacteria active in sausage fermentation has been found to efficiently inhibit *S. aureus* by means of bacteriocins (5). *S. aureus* constitutes a risk to health only after growth

in a food product to levels of about 10^5 or 10^7 cells g^{-1} (5). Production of toxins is limited at a_w 0.87 for toxin type A, 0.90 for type B, and 0.94 for type C (18). Thus, drying in the southern technology in which a_w varies from 0.90 to 0.93 after fermentation (10) does not inhibit all toxin production by *S. aureus*.

Listeria monocytogenes is more frequently involved in sporadic cases of food poisoning. It is ubiquitous and can establish in plants as an in-house bacterium (23–25). It has been reported that as many as 68% of environmental samples in a curing plant were positive and 17% remained positive after cleaning (26). Recent European investigations have reported 12–16% *Listeria*-positive samples in fermented industrial products (27,28). There is no epidemiological evidence for the involvement of fermented sausages in recent outbreaks of listeriosis, and the International Commission on Microbiological Specification for Foods recommends that up to 100 cells of *L. monocytogenes* per gram of fermented meat products be tolerated (27,28). However, *L. monocytogenes* is slowly inhibited during sausage fermentation, and it is desirable to eliminate this microorganism from raw ready-to-eat meat products. *L. monocytogenes* can grow at pH levels between 4.6 and 9.6 and cannot grow below a_w 0.90 (28). This means that drying during ripening limits its growth. Using lactic acid bacteria that produce bacteriocins active against *L. monocytogenes* can reduce the level of this pathogen in fermented sausages by about one or two log cycles compared to non–bacteriocin-producing bacteria with similar souring activity (5). This applies to various types of fermented sausages (Table 3). However, no reduction higher than two logs was noticed, which can be explained by interactions between bacteriocins and meat or fat materials (29).

Cattle are the main reservoir of enterohemorrhagic *Escherichia coli* (EHEC) but pigs are also implicated (30–32). Foods commonly associated with infection are of animal origin and include raw minced meat and fermented meats (33,34). The consumption of contaminated salami slices was responsible for an episode of food poisoning in 1994 in the United States. Indeed, *E. coli* O157:H7 has been reported to survive during manufacturing of sausages (35,36) and during storage of slices (37,38). Lactic acid bacteria can accelerate the destruction of EHEC by lowering the pH and thus facilitating drying.

B. Toxic Compounds: Biogenic Amines

Biogenic amines such as histamine, tyramine, cadaverine, and putrescine are low-molecular-weight compounds that are produced from amino acids by microbial decarboxylation. Some

Table 3 Effect of Various Bacteriocinogenic Lactic Acid Bacteria on *Listeria* in Fermented Sausages

Type of sausage	Bacteriocinogenic strains	Decimal reduction of *Listeria* count[a]
Fresh (low acid)	*L. sakei* Lb706	1[b]
Dried German type	*L. sakei* and *L. curvatus* strains	0–2
Spanish type	*L. sakei* and *L. curvatus* strains	0–2
	Enterococcus faecium	No reduction
Italian type	*L. plantarum* MCS	Slight effect on survival
US type	*P. acidilactici*	0.5–2.5

[a] comparison with a control strain nonbacteriocinogenic.
[b] regrowth at pH > 6.0.
Source: Ref. 5.

biogenic amines are hazardous to health due to their vasoactive and/or psychoactive properties (39). Biogenic amines have repetitively been shown to be present in meat products from both northern and southern technology (Table 4) (40–42). However, in most samples, the quantity of biogenic amines is lower in northern sausages than in southern sausages (Table 4). As an example, if we compare the level of biogenic amines in two northern and two southern products studied by the same authors (6,43), it varied from 30 to 130 mg/kg in northern products versus 298 to 371 mg/kg in southern products. The main amine found is tyramine and its concentration in southern product reaches the threshold of 100–800 mg/kg of food, which is considered unsafe by Ten Brink et al. (44). The second amine found in both technologies is putrescine, whose limit has not been laid down in food. Putrescine is not associated with symptoms but it can combine with nitrites to form carcinogenic nitrosamines (45).

Enterobacteriaceae and *Pseudomonas* are frequently isolated from raw materials but also from sausages, and this spoilage flora can produce biogenic amines, especially during the first days of fermentation (46,47). Enterobacteriaceae produce histamine and cadaverine and *Pseudomonas* produces putrescine (48). Usually this spoilage flora is less inhibited by the southern technology than by the northern one. *Enterococcus* spp. mentioned as subdominant bacteria in meat products (7) produce biogenic amines and particularly high levels of tyramine (49). The hygienic quality of raw materials appears to be one of the main factors affecting biogenic amine formation in dry fermented sausages (44,50,51). Some strains of lactic acid bacteria, *Staphylococcus* and *Kocuria*, are also able to produce amines (52,53).

Table 4 Content of Biogenic Amines in Different Products from Northern and Southern Technologies

Products	TRYP	PHE	PUT	CAD	HIS	TYR	Total
Northern sausages							
Belgian mg/kg DM[a]	nd	10	150	20	40	370	590
Norwegian mg/kg DM[a]	nd	nd	10	nd	2	10	22
Dutch mg/kg DM[a]	nd	10	50	60	10	110	240
Belgian mg/kg[b]	17	3	33	nd	1	76	130
Norway mg/kg[b]	9	6	1	1	1	12	30
Southern sausages							
French mg/kg DM[a]	nd	10	109	170	140	560	989
Spanish salchichon mg/kg DM[a]	30	20	130	30	30	30	270
Belgian mg/kg[b]	39	27	33	5	18	176	298
Italian mg/kg[b]	19	39	125	1	nd	187	371
Italian soppressata mg/kg[c]	nd	nd	128	7	71	206	412
Italian salsiccia mg/kg[c]	nd	nd	34	39	nd	339	412

TRYP: Tryptamine; PHE: Phenylethylamine; PUT: Putrescine; CAD: Cadaverine; HIS: Histamine; TYR: Tyramine; DM: dry matter.

[a] From Ref. 137.

[b] From Ref. 43

[c] From Ref. 42.

The levels of biogenic amines in sausages can be controlled by reducing the microbial contamination of the raw material via high acidification and by inoculation with starters capable of degrading biogenic amines. Recent results show that *Kocuria varians*, *Staphylococcus xylosus*, *Lactobacillus casei*, and *L. plantarum* strains isolated from dry fermented sausages degrade tyramine under laboratory conditions (54,55).

C. Pathogenic Molds/Mycotoxins in Southern Products

In the northern process, smoking inhibits the development of molds. In contrast, in the southern process, some traditional producers still rely on the "house" flora to provide a natural inoculum for mold coating and ripening of dry fermented sausages, although due to increasing concerns about safety, the use of specific fungal starter cultures is likely to become more widespread. The spontaneous and heterogeneous nature of the "house" mycoflora can lead to faulty products and in particular to the production of mycotoxins in the product (56,57). Andersen (58) conducted an extensive survey of the mycoflora of mold-fermented sausages in Europe and found that *Penicillium* constitutes 96% of the mycoflora with species of *Aspergillus*, *Cladosporium*, *Eurotium*, *Mucor*, and *Wallemia* forming only a minor component. *Penicillium nalgiovense* formed 50% of the mycoflora with *Penicillium chrysogenum*, *Penicillium verrucosum*, *Penicillium oxalicum*, and *Penicillium commune* representing 10%, 5%, 3%, and 3% of the mycoflora, respectively. In Spanish fermented sausages, *P. commune* and *Penicillium olsonii* are the dominant species (59). None of the *P. olsonii* isolates produce toxic compounds, whereas all *P. commune* isolates produce cyclopiazonic acid (59). Molds have been isolated from red and black peppers commonly added to various kinds of Spanish sausages (60). From this study, 19% of the isolates belong to the *Aspergillus flavus* group and 36% of this group produce aflatoxin. Experimental contamination of Spanish sausages by these aflatoxigenic strains of molds results in the presence of aflatoxin only on the skin of the sausages (60). As many *Aspergillus* and *Penicillium* species are capable of producing mycotoxins, it is very important to select safe starters (56,57). Nontoxinogenic strains of *P. nalgiovense* and *P. chrysogenum* are developed as commercial starter cultures.

IV. SENSORY QUALITIES OF SOUTHERN- AND NORTHERN-TYPE SAUSAGES

Among the sensory characteristics, flavor is the most important. Whereas the appearance (color) and texture initiate purchase or rejection of the product, flavor is the feature that convinces the consumer to buy the product again.

A. Color

The typical cured-meat color is associated with the formation of nitrosomyoglobin, which results from a series of reactions involving the formation of nitrogen oxide (NO) and its reaction with myoglobin producing nitrosylated pigments, which yield a red color (61).

In the Mediterranean process, the substrate added to produce NO is mainly nitrate, whereas in the northern process, nitrite is added as coloring salt. Use of nitrate involves bacterial reduction to nitrite. It is carried out by coccal gram-positive bacteria but their nitrate reductases are inhibited by pH below 5.2 (62) (Table 5). Nitrite acts as a very

Table 5 Action of Starter Cultures in Dry Sausage

Characteristics	Mode of action	Microorganisms involved			
		Lactic acid bacteria	Cocci Gram-positive	Yeasts	Molds
Preservation	pH drop	+++	–	–	–
	Nitrate reduction	–	+++	–	–
	Bacteriocin	++	–	–	–
	Amines degradation	+	+		–
	Prevention of mycotoxin	–	–	–	+++
Color	Nitrate reduction	–	+++	–	–
	pH drop	+++	–	–	–
	H_2O_2 breakdown	–	++	–	+
Texture	pH drop	+++	–	–	–
Flavor	Acid production	+++	–	–	–
	Protein breakdown	+	+	+	++
	Fat breakdown	–	+	+	++
	Antioxidant properties	–	++	+	++
	Amino acid catabolism	(+)	++		
	Fatty acid catabolism	–	++		+
Condition of surface	Apperance	–	–	–	+++
	Protection from drying out	–	–	–	+++
	Protection from O_2 and light	–	–	+	+++

+++: very important; ++: important; +: noticeable effect; (+): weak effect; –: no effect

reactive oxidant and is reduced to NO immediately after preparation of the sausage mix. The reduction of nitrite to NO is favored by the acidification caused by lactic acid bacteria (Table 5). Color, tone, and intensity of products vary perceptibly according to whether they are cured with nitrite or nitrate (63).

Discoloration of cured meat can be observed by the formation of peroxide. This default can be avoided by the catalase activity of coccal gram-positive bacteria that protect the color (64) (Table 5).

B. Texture

During comminution, addition of salt solubilizes muscle proteins, which coagulate and form a gel surrounding lard and meat particles because of the acidification brought about by lactic acid bacteria (Table 5). The pH required for coagulation increases with increasing salt concentration and is 5.3 at the often-used salt concentration of 3% (7). Coagulation by acidification is associated with the release of water at the isoelectric point of the meat proteins (pH 5.3). The gel formed by coagulation is further stabilized by the release of water, and it forms a matrix surrounding fat and meat particles, which determine sausage texture. The drop in pH affects texture development during fermentation, whereas further

texture development during drying is only affected by the loss of water. Numerous factors affect the interrelated rates of both acidification and drying. Lactic acid bacteria are known to increase the rates of acidification and drying and thus of texture development (65). Increasing sausage diameter clearly decreases the rate of drying and thus the rate of hardness development (66).

C. Flavor

A large variety of compounds are likely to contribute to the desired (and undesired) aroma and taste of fermented sausages. Some of them are added to the sausage mix as such (salt, constituents of spices, and smoke), but abiotic reactions, and tissue or microbial enzymes are responsible for the formation of other compounds during ripening. Although taste and smell should be seen as integrated sensations leading to flavor, it is usual to differentiate between chemical compounds determining taste and aroma, respectively.

D. Taste

Nonvolatile compounds contribute to the taste of meat products. They mainly consist of inorganic salts, nucleotide metabolites, sugars, acids, amino acids, and peptides and are often present in relatively high quantity (61).

1. Acids

The acid taste is an important component of the overall taste of fermented meat products; it is sought in the northern process but may be rejected in the southern process. It is positively correlated with lactate and acetate contents (5,67,68). Too much acid production has been claimed to lead to undesirable "sour", "prickly," and "astringent" off-flavors (69). Ramihone et al. (70) associate acidity with excessive amounts of D-lactic acid.

Lactic and acetic acids are produced mainly by carbohydrate fermentation by lactic acid bacteria. Their production depends on the type and concentration of carbohydrates added to the meat batter, on technological factors, and particularly on the strains of lactic acid bacteria (71). In fact, acidification rate constitutes a major criterion of selection for lactic acid bacteria.

Other short-chain organic acids have been reported in fermented sausages; their concentrations are 100- to 1000-fold lower than those of acetic acid (68,72,73). Given the low odor and taste levels (low ppm range) of many of these acids (74), the possibility that these compounds play a role in flavor development cannot be discarded (68).

The level of short-chain organic acids including acetic acid but not lactic acid is very different in southern and northern products (73). It varies from 8.9 to 22 mg/kg DM in French, Italian, and Spanish sausages versus 79 mg/kg DM in German salami (73). The levels of lactate and acetate in northern products are higher than in southern products, as mentioned in Sec. II.

2. Peptides and Amino Acids

Peptides and amino acids belong to the nonvolatile fraction of dry sausages. Their contribution to the taste of dry sausages has not yet been clearly elucidated. Free amino acids and some peptides have some sweetness, bitterness, sourness, saltiness, and umami taste with generally high threshold (>50 mg/L for glutamate, up to 2000 mg/L for leucine) (61,75). Van

Hoye et al. (76) and Dierickx (77) show a clear correlation between free amino acid and peptide concentrations in sausage and taste descriptors such as spicy, beefy, sweet, bitter, and astringent. Henricksen and Stahnke (78) have identified amino acids and peptides that could contribute to the taste. However, a strong and rapid increase in amino acid concentration following proteinase addition has no marked impact on the final flavor but accelerates maturation (43,79–81). Similarly, products that have a different amino acid composition after inoculation of *Pediococcus pentosaceus* or *Pediococcus acidilactici* in association with *Kocuria varians* have identical sensory characteristics (82).

In dry sausages, myofibrillar proteins are the most degraded proteins. Their breakdown into peptides is due to endogenous enzymes because the use of antibiotics and paucimicrobial meat incubations does not reduce degradation of actin, myosin, or troponin (83,84). Bacterial starters have a low proteolytic activity on myofibrillar proteins (85) but they contribute to the hydrolysis of sarcoplasmic proteins (86–90). Lactic acid bacteria indirectly contribute to proteolysis by reducing the pH, which increases cathepsin D activity (83,84). Proteolysis of myofibrillar proteins is greatly controlled by pH.

In dry sausages, breakdown of polypeptides into smaller peptides and free amino acids is of either endogenous (60%) or microbial origin (40%) (83). Thereby, sausages containing antibiotics have lower amino acid concentrations than those inoculated with *Staphylococcus* and *Kocuria* (91,92). Peptidase activities are reported in lactic acid bacteria (82,88–90,93,94). However, at pH values below 6, such peptidase activities are low (93).

Proteolysis is very different in northern and southern processes because pH is a very important parameter in proteolysis. This means that acidic sausages produced according to the northern process are characterized by a clear degradation of actin and myosin and consequently a high peptide formation but low ammonia production. Nonacidic sausages produced in southern countries show no or a weak breakdown of myosin and actin and thus a low production of peptides and free amino acids. These different processes clearly have an impact on proteinase as well as peptidase activity (6,68,83).

3. Fatty Acids

Lipolysis in dry sausages releases free fatty acids with chain length between 16 and 18 carbon atoms (95). Their direct role in flavor has not been demonstrated yet. Short chain fatty acids have sour tastes but their sensory characteristics diminish with increasing chain length (74). In fact, fatty acids with chain length greater than 12 carbon atoms have no taste or smell (96). Addition of exogenous lipases leads to a faster and more intense lipolytic process during ripening of dry fermented sausages; however, sensorial improvement of these products is not as obvious as expected (97,98). What is considered to be of greatest significance for flavor is the role of these fatty acids and of unsaturated fatty acids as precursors of smaller-molecular-weight compounds with highly characteristic flavor properties (68).

It is now well established that lipolysis in dry sausages is, to a great extent, of endogenous origin (84,99). There are two types of endogenous lipases: triglyceride lipases present in the muscle and in the adipose tissue, which act on triglycerides, and phospholipases, which hydrolyze phospholipids and whose optimal pH of 5.5 is close to that of sausages (100–102). The importance of endogenous lipolysis has been demonstrated by manufacturing aseptically or in paucimicrobial sausages inoculated by micrococci or staphylococci and comparing the increase in the level of fatty acids (101,102) and also by manufacturing sausages using antibiotics (99). According to Hierro et al (102), lipolysis by endogenous enzymes accounts for more than 60% of total free fatty acid release. Similarly, Johansson (103) considers that about 30% of lipolysis come from the lipolytic strain of

Staphylococcus xylosus inoculated in a sterile mixture of fat and pork lean. Some lipolytic bacteria such as *Staphylococcus* and *Kocuria* are described, nonetheless, their activity is very low at pH 5.0, which is the pH of northern sausages, whereas at a pH near 6.0 (final pH of some Mediterranean sausages, some lipolytic activities are measured (104–106).

Free fatty acid content is obviously determined by both length of ripening period and raw materials used. A higher level of total free fatty acids is noticed in the southern process: 37 mg/g versus 27 mg/g in the northern process (6).

E. Aroma

Odor or aroma is by far the most important component of flavor because of the high sensitivity of nasal receptors to the numerous volatile components released during chewing and ingestion. The number of aroma compounds derived from spices and smoking (northern-type sausages) exceeds that of compounds derived from metabolism (107). It is clear that northern products are characterized by a smoked flavor in relation to numerous compounds such as phenols, methoxyphenols, and cyclic ketones (108,109). Garlic addition to sausages result in high levels of sulfur compounds with strong aroma. Similarly, addition of pepper leads to the presence of many terpenes but does not contribute greatly to the overall odor (107). Beside the volatile compounds originating from spices or smoke, compounds derived from the further metabolism of carbohydrates, amino acids, and fatty acids are considered to be very important for the specific flavor (110–112). The level of some catabolites varies with the process.

1. Volatile Compounds Arising from Carbohydrates

Carbohydrate fermentation mainly results in the production of lactic and acetic acids as mentioned above. Acetic acid is involved in the acidic taste but also in the vinegar odor of sausages. This odor note is higher in northern products (107,109). Sugar fermentation also results in the production of polyfunctional ketones with a strong buttery smell (diacetyl, acetoin). The level of diacetyl and acetoin can reach high values in northern products, 1300–1400 ng/g of sausages (108) and from 3052 to 4246 nmol/kg of sausages (6,109), whereas it is about 500 ng/g of sausages (108) and varies from 500 to 681 nmol/kg (6,109) in southern sausages. These ketones are produced by lactic acid bacteria (113) and staphylococci, particularly *Staphylococcus saprophyticus* and *Staphylococcus warneri* (110,114).

2. Volatile Compounds Arising from Amino Acids

Amino acids can be broken down into amines, ammonia, or aromatic compounds.

Volatile amines have been little studied in fermented products, although biogenic amines are often detected and have to be avoided because of their toxicity (see Sec. III.B). Among these amines, only putrescine and cadaverine have unpleasant odors with high threshold values, and it is doubtful whether the amounts produced are sufficient to influence taste or smell. Given its contribution to the increase in pH observed during drying, ammonia could also influence the sensory properties of compounds with ionizable groups (68). Ammonia production is particularly evident in sausages with a long drying phase, such as southern-type sausages (6,115).

The breakdown of branched-chain amino acids (leucine, isoleucine, valine) or phenylalanine results into aldehydes, alcohols, and acids with odors detected at very low threshold values (112). Thus methyl alcohols with fermented fruity odors, methyl aldehydes with

malty and bit fruity odors, and methyl acids with cheesy and sweaty socks odors contribute to the dry sausage aroma (110,111,114,116). Phenylacetaldehyde with floral odor and benzaldehyde with almond and acre odors are also detected in sausage (112). The degradation of methionine, which leads to methanethiol and dimethylsulfide, with cabbage and putrid odors, must be avoided (68,109).

Catabolites of branched chain amino acids are found in both types of sausages. The relative amounts of these catabolites are highly variable. According to van Opstaele and Dirinck (108), the levels of aldehydes and branched alcohols are higher in French and Spanish sausages than in Belgian sausages. The level of methyl acids is higher in French products than in Belgian ones, and acids are absent in Spanish products (108). According to Schmidt and Berger (107), methyl acids are present in much higher levels in German sausages compared to Mediterranean sausages (France, Spain, and Italy).

The catabolism of amino acids could involve the Strecker reaction (117,118) as well as bacterial catabolism. The production of volatile compounds arising from amino acids in sausages is modulated by the inoculated flora and, in particular, by staphylococci (110,114). In fact, in sausages inoculated with *Staphylococcus carnosus*, the desorption of 3-methyl butanol, 3-methyl butanal, and 3-methyl butanoic acid is larger than in those inoculated with *Staphylococcus warneri* and *Staphylococcus saprophyticus*. The 3-methyl butanal plays an important role in the cured meat aroma of sausages inoculated with *S. xylosus* (111). Hinrichsen et al. (119) associate the detection of 3-methyl butanal with the presence of *S. warneri*, *S. saprophyticus*, and *S. xylosus* in bacon. Under laboratory conditions, *S. carnosus* produces large amounts of 3-methyl butanoic acid but also 3-methyl butanal and 3-methyl butanol from leucine (120,121). Lactic acid bacteria have a restricted aromatic potential. Under laboratory conditions, *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, and *Pediococcus acidilactici* only weakly degrade leucine, mainly into α-ketoisocaproate, a molecule that is not very odorous (121).

3. Volatile Compounds Arising from Fatty Acids

Fatty acid oxidation results in numerous compounds, which belong to six families: alkanes, alkenes, aldehydes, alcohols, ketones, and acids. Although the production of these compounds in sausages is low, on the order of parts per million, their low sensory threshold (except for alkanes and alkenes, which are odorless) means that they have a real effect (72, 122). In southern sausages without spices, they represent 60% of the volatile fraction (110).

Results of aldehyde analysis show that Mediterranean sausages obviously contain higher amounts of 6–10 carbon straight-chain aldehydes, with a marked difference for hexanal, a typical product of linoleic acid oxidation (6,107,108). They have flavor thresholds of 5 to 60 ppb, and flavors are variously described as green, metallic, fruity, fatty, and rancid (68). Ketones from 2-pentanone to 2-nonanone with fruity, musty, cheesy odours and alcohols (saturated and unsaturated) from 6 to 8 carbon atoms with fruity, green, and mushroom odors are found in higher amounts in southern products than in northern ones (107–109).

Oxidation of fatty acids can be chemical (peroxidation) or enzymatic (β-oxidation) (122). Peroxidation of mainly unsaturated fatty acids is a radical process that leads to the synthesis of hydroperoxides, which are oxidation products but also substrates for the formation of other radicals (RO, ROO, etc.) that propagate the chain reactions. The condensation of two free radicals halts the process and generates secondary oxidation products (aldehydes, alkanes, etc.) (61,123). Oxidation reactions are affected by many factors, such as oxygen content (diameter of sausages), the presence of pro-oxidative compounds (NaCl, metals) or anti-oxidative compounds (nitrite, spices), and the amount of unsaturated lipids

(122,124). In sausages, microorganisms play a vital role in the regulation of oxidation. Thereby, model sausages inoculated with *S. carnosus* and *S. xylosus* have lower alkane and aldehyde desorption levels than those inoculated with *S. warneri* and *S. saprophyticus* (110,114). The latter are characterized by a rancid aroma (114). Under laboratory conditions, *S. saprophyticus* and *S. warneri*, but above all *S. xylosus* and *S. carnosus*, limit the oxidation of linoleic and linolenic unsaturated fatty acids (125). The catalase and superoxide dismutase (SOD) activities of *S. carnosus* have been characterized (126). In *S. xylosus*, Barrière et al. (127,128) used mutants deficient in SOD or catalase activity to show that these enzymes contribute to limiting the oxidation of unsaturated fatty acids.

The presence of ketones in sausages is associated with the inoculation of *S. carnosus* or *S. xylosus* (110,114). These ketones may be the result of incomplete fatty acid β-oxidation. Usually, β-oxidation degrades saturated fatty acids into acetic acid by successively eliminating acetyl CoA groups. However, intermediate CoA esters can be freed; they are successively converted into β-ketoacid via thioesterase activity and then into methyl-ketone and secondary alcohol via decarboxylase and dehydrogenase action. The mechanism of ketone formation is described for molds, and because molds are found in southern products, this can partly explain the higher level of ketones in these products (129). Concerning *S. carnosus*, Engelvin et al. (130) have shown that intermediates were freed during the β-oxidation cycle. They also mentioned the thioesterase activity of this strain. Fadda et al. (129,131) have shown that *S. carnosus* has β-decarboxylase activity. All these results suggest that *S. carnosus* may produce ketones via this pathway.

4. Esters

Esters are present in fermented meat products and their aromatic characteristics contribute to the fruity note of the products (114,132). Most of the esters in sausages are ethyl esters, their production depending on the presence of ethanol and different acids (from two to eight carbon atoms) as well as on technological factors and microorganisms.

According to van Opstaele and Dirinck (108), sausages of the southern type are characterized by a higher proportion of ethyl esters. For Schmidt and Berger (107), it is also true for two types of Mediterranean products, the French and the Italian ones, whereas Spanish and German products have the same level of esters.

Esters could be of chemical origin because they are found in dry raw ham with a low bacterial count. But they can also be of bacterial origin. In laboratory media, some staphylococci have esterase activities capable of hydrolyzing and forming ethyl esters (133,134). In sausages, esters are associated with the presence of *S. xylosus* or *S. carnosus* strains (114,132).

Finally, the large analytical differences between the two types of sausages are reflected in the results of sensory analyses. German sausages exhibit a strong buttery and sour odour, but low levels of spicy and fruity notes (107). Mediterranean sausages (French, Spanish, Italian) are characterized by fruity, sweet odors with medium scores for buttery, sour, and pungent attributes (107). In another study, the panel characterized northern products as more acid and southern products as more mature (6,109).

V. CONCLUSION

It is of course schematic to consider only two types of dry fermented sausages because there are as many sausage types as there are countries or regions. But considering these two

categories makes it possible to establish some specific characteristics. In northern products, rapid acidification by lactic acid bacteria to a final pH below 5 as well as smoking ensure safety, improve shelf life, and contribute largely to the sensory quality. In southern products, acidification reaches a final pH above 5 and safety and shelf life are mainly ensured by drying and lowered water activity. Under these conditions, development of coccal gram-positive bacteria can be favored and these contribute to the sensory quality of these products.

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24
Mold-Ripened Sausages

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I. INTRODUCTION

Ever since the realization that one of our most important foods, meat, cannot be left "as is" because in some hours or some days—depending on the ambient temperature—it spoils (i.e., cannot be eaten), human beings came up with ways of "preservation." Of these preservation techniques, drying and later fermentation and drying are probably the most ancient ones. In early ages, meat pieces were dried (and smoked) in caves above open fires, which "technology" must have been remembered much later, too, when animals were domesticated and raised as a source of meat (and a source of milk as well as leather and fur). At that time, open fires existed in the nomads' tents. At a later stage, some hundreds to some thousands of years ago, whole pieces of killed domesticated animals were rubbed with salt and spices, or meat and fat cut into small pieces were mixed with salt and spices, and were dried, making use of the animals' intestines. This way it was possible to supply humankind with food of animal origin all year round; and in the past centuries, fermentation and drying has become an art—the manufacturing of high-quality meat products (1). A real breakthrough in fermented dried meat product manufacturing happened only about 50 years ago, when Niinivaara and Niven discovered the great potential of controlled fermentation. These two excellent scientists revolutionized fermented meat manufacturing, a field of meat processing which has developed ever since both in theory and practice. Importance of this starter culture technology is clearly shown by its benefits: a rapid turnover, a typical and consistent pleasant aroma and taste as well as good safety record in comparison to natural fermentation, a process in which the ever-changing microflora of raw materials may metabolize the ingredients thus affecting the quality of a product mainly because of shorter ripening time. It has to be emphasized, though, that there are traditional technologies (e.g., Italian and Hungarian salami, Iberian and Parma ham) in which excellent sensory quality and safety records can be achieved; this is ensured, in addition to (curing) salt, by a long ripening-drying time at low temperature, relying only on these selecting effects: allowing growth of desired microorganisms yet inhibiting growth of undesired members of the natural microflora. As a matter of fact, the safety record of mold-ripened meat products—characterized by long drying times and low a_w-values—is better than that for short-ripened products because of low water activity, which is a reliable hurdle alone, inhibiting pathogenic growth. (In the case of rapid fermentation technologies, metabolic activity of

surface mold may cause breakdown of lactic acid, thus raising pH and allowing growth of pathogenic and spoilage microorganisms.) As far as food safety is concerned, possible presence and activity of toxin-producing molds also has to be dealt with in the case of mold-ripened meat products.

Mold-ripened products can be divided basically into two groups:

Those made of intact meat cuts: i.e., different types of hams
Those made of comminuted meat and fat stuffed into (natural or artificial) casing: i.e., different types of sausages

This difference in appearance requires basic difference in technology. In this chapter, production of sausage will be described.

II. MOLD-RIPENED SAUSAGES

These products are raw-fermented and dried; mostly, but not always, manufactured with starters in the sausage mix (micrococci, sometimes lactobacilli and yeasts, and—on the surface—atoxigenic mold starters); ripened and dried mostly, if not always, for a longer period of time (several months). Because of chemical changes due to longer ripening-drying times, final pH is usually higher (pH > 5.5), even if pH value after incubation was lower. For this reason, it is of vital importance, from the food safety point of view, that final a_w-value be low enough, which requirement is fulfilled by a long drying period.

Some typical mold-ripened products are French, Hungarian, Italian, California and Yugoslavian salami; of these, the Hungarian product is manufactured traditionally, without application of starter cultures.

III. TECHNOLOGY

A. Raw Material

High-quality product can be manufactured only from high-quality ingredients—that is, from hygienically processed raw material with good technological and sensoric attributes produced under good manufacturing practices. Structure and texture, as well as sensory value, are influenced (2) by the following:

Type of meat (pork, beef, etc.)
Type of fat (saturated-to-unsaturated fatty acid ratio)
Type of fat tissue (marbling, back fat)
Meat-to-fat ratio in sausage batter
Particle size
Chopping, grinding, stuffing temperature
Type and concentration of seasonings
Casing diameter
Pattern of pH changes
Salt concentration
Microflora (starters or spontaneous)
Final water activity

As for the technological quality of meat, the "drier" muscle of sows—with higher intramuscular fat content—is more suitable than the lean muscle of young animals if a low-water-

activity dry salami is the aim, achieved through a long ripening. Meat with higher fat and lower moisture content allows for better control of drying; also, less shrinking of sausage diameter occurs, which gives a more attractive product.

Presence of pale, soft, exudative (PSE) and dark, firm, dry (DFD) meat is less of a problem because of the low incidence of such types, their presence is compensated for by comminution and mixing with normal meat. Formation of texture greatly depends on pH value: when lactic acid is produced by starter cultures, gelifying a protein solution containing meat and fat particles, a firm texture and sliceability is attained more rapidly than when the sausage pH is far from the isoelectric point of muscle. With a higher sausage pH (low acidity), a significant rise in salt concentration (through drying) is the only factor responsible for gelifying the protein solution and for sliceability, which is the result of the gel structure of the protein matrix.

B. Prefreezing and Comminution Techniques

Depending on the comminution technique (chopping or grinding) prefreezing either is a must—or can be omitted. If raw material is ground (e.g., in Italy), no prefreezing is needed; chilling is adequate. But if chopping (bowl chopper) is applied (e.g., in Germany, Hungary), prefreezing of fat (-5 to $-7^{\circ}C$) and meat cannot be avoided; otherwise, smearing of fat particles during chopping and stuffing causes loss of sensory quality and drying failure and leads to hygienic risk, too. Even if comminution is done properly, adequate stuffing is needed to avoid smearing of sausage batter; in addition to the appropriate temperature, the right choice of stuffing machine is of crucial importance.

C. Fermentation

In a broader sense, we may consider fermentation not only as the breakdown of tissue and added carbohydrate but as consisting of other metabolic activities as well; yet fermentation here refers mainly to the first couple of days: the long-lasting process afterwards that results in the aroma richness typical for this type of product is considered ripening.

D. Use of Starter Culture

In long-ripened sausages, just as in short-ripened ones, lactic starters reduce pH and contribute to aroma; micrococci play a role mainly in color and aroma formation. Because micrococci require higher pH, the pH drop caused by lactobacilli may retard their growth. Preripening (keeping the sausage at $5^{\circ}C$ for some days) can give micrococci a chance to grow before pH drop (3).

Depending on whether starter cultures (lactic starters alone or in combination with micrococci) are used or not, initial temperature (incubation) is basically different. In the presence of starter culture the temperature has to be adjusted to the microorganisms' need, usually below $30^{\circ}C$ ($18–24^{\circ}C$) in Europe and above $30^{\circ}C$ ($32–37^{\circ}C$) in the United States. The presence and metabolic activity of the starter culture contributes to product safety through its inhibitory effect against undesired microbes. But if no starter culture is added to the sausage batter, another inhibiting factor, low temperature, has to be relied on. This low temperature (usually below $10–12^{\circ}C$), together with common and curing salt, ensure sufficient protection against growth of pathogenic and spoilage microorganisms until adequately reduced water activity ensures inhibition also at higher temperatures.

Types and application of starter cultures are discussed in several publications; some of them are mentioned here (4–6).

E. Color Formation

Raw fermented sausages are usually manufactured with curing salt, in earlier times and in some cases also today with KNO_3, but mostly with nitrite ($NaNO_2$), or a mixture is applied. If nitrate is used it has to be reduced by microorganisms to nitrite, which then reacts with myoglobin to give nitroso-myoglobin. The more stable pigment is formed most likely by denaturation through increasing salt concentration during drying. Dry sausage can also be manufactured with common salt only, yet the microbiological risk is higher if the antibacterial effect of nitrite cannot be relied on. This is even more pronounced if for health purposes sodium content is reduced. In this case, safety requirements can be met only if other a_w-reducing substances are applied. In the absence of curing salt, color stability of sausage is reduced, but intensive smoking improves it to some extent because NO-compounds from smoke deposit and diffuse inside (7).

F. Smoking and Mold Growth

Most mold-ripened sausages are not smoked; this is explained by the long traditions that offer some advantages, mainly in recent decades since artificial inoculation of the surface by starter molds like *P. nalgiovense*, *P. chrysogenum*, and *P. cemembertii* (5) has been applied. Starter molds are sensitive to smoke constituents in general, and this retards mold growth, resulting in scarce and uneven growth. There are, nevertheless, mold-ripened sausage technologies in which after intensive smoking, surface inoculation with mold starters is applied, or spontaneous mold growth covers the surface (e.g., Hungarian salami). For even growth on the whole surface of this latter type, sporadic colonies are spread by brushing. Smoking has manifold advantages:

Inhibition of growth of yeasts, (slime-producing) micrococci, and undesired, toxigenic molds
Strong antioxidative effect retarding rancidity
Significant positive influence on sensory characteristics

As a result, smoked products are significantly different in taste and aroma.

Growth of molds, be it inoculated or spontaneous, has to be supported by suitable temperature and relative humidity parameters. This is a process with risk involved, because with too low a temperature and relative humidity, mold growth is retarded and undesired microbes might grow instead; on the other hand, with too high a temperature and relative humidity, pathogenic and/or spoilage microorganisms are able to grow inside the sausage, mainly if a_w is not low enough, and molds metabolize lactate, which allows growth and toxin production of staphylococci (8). Consequently, strict control of these parameters in accordance with the requirement of mold applied is of vital importance.

If mold growth is well supported, an even mold cover on the surface is formed that sticks well to the product and remains intact during handling, unless abuse occurs during storage.

A well-developed mold layer plays an important role during further technological steps and also during storage in retail and in households. After mold layer is completed, relative humidity, and in some cases also temperature, is decreased and drying is continued. Thanks to this mold layer, moisture loss in sausages is more balanced than without mold;

consequently, risk of too-rapid drying causing case hardening is significantly less. In addition, oxidative rancidity is also retarded, not only by less influence of oxygen but also by light protection, chiefly in retail.

G. Ripening-Drying

Ripening is a most complicated process controlled only to some extent by technologists and completed mostly by tissue enzymes and by metabolic activity of inoculated and/or spontaneous microorganisms.

Intentional means for control are application of starter culture, type and concentration of additives (intrinsic factors), and mainly adjustment of temperature, relative humidity, and air velocity in function of time (extrinsic factors), controlling in this way the drying rate (6). Uniform air distribution is a precondition for uniform and consistent quality. Contrary to short-fermented sausages, in which to some extent also tissue enzymes and definitely bacterial enzymes are active in the final product, too, this process can slow down or even stop after a long drying period of several months, resulting in a_w-values as low as 0.85.

IV. CHANGES DURING RIPENING DRYING; AROMA FORMATION

Controlling temperature and drying rate plays a decisive role in food safety by inhibiting growth of undesired microbes but this control is needed also for a consistent sensory quality. As a result of drying (i.e., a_w-reduction), less resistant microorganisms gradually disappear (members of Enterobacteriaceae, pseudomonads, bacilli, etc.); less sensitive ones (micrococci, staphylococci, enterococci, listeriae, EHEC, etc.) may survive for a longer period of time. In the final product of long-ripened dried sausages, spoilage microflora has practically no chance to grow and cause deterioration. The situation is somewhat similar with pathogenic microorganisms, yet their very presence is not accepted by food authorities requiring sometimes zero tolerance—a standard that is hard to support scientifically.

In order to meet safety requirements, a complex strategy has to be followed by adding and/or supporting growth of useful microorganisms in the hygienically produced sausage and by controlling ripening-drying parameters, thus favoring growth of useful microorganisms and inhibiting growth of undesired ones, as discussed above. While doing so, not only are safety requirements met but also aroma formation is contributed to.

Starter cultures and bacteriocin-producing microorganisms can help in inhibiting pathogenic microbes (8–12) where inhibition of staphylococci and listeriae is desired mostly because these bacteria are difficult to combat, a result of their resistance to low a_w, the main controlling factor in long dried sausages.

It should be kept in mind that bacteriocin producers are in general more effective under laboratory conditions on media than in a food matrix (12).

Unlike in dried ham, tissue endoenzymes in long-dried sausages play a less important role in aroma formation, and they are active mainly in the first part of ripening (13). In long-dried sausages, the metabolic activity of microorganisms contributes more to aroma but other factors— such as spices, lipolysis, etc.—may be involved in this complex process, too.

As mentioned already, smoking and pH changes (low- and high-acid sausages) do influence aroma characteristics, but the types and concentrations of spices used determine aroma even more (14). In this respect, spices that can be evenly distributed in sausage batter and react immediately with meat and fat matrix have a much more pronounced impact on

aroma characteristics than occurs in long-ripened hams, where spices are generally not applied or they are rubbed on the muscle surface only. Natural spices in sausages influence aroma as well as safety indirectly, too, by enhancing metabolic activity of starter cultures (11,15).

As for taste and aroma formation by microbial metabolism, the breakdown of proteins, peptides, lipids, and carbohydrates will be discussed next.

Depending on application of lactic starters and addition of carbohydrate, lactic acid is produced through homofermentation, reducing the pH level (9,16). Concentration of D-lactate may be 5 times higher in high-acid sausages than in low-acid products (17). At a later stage, pH also increases in sausages with lactic starters; this is related to the formation of ammonia and some amino acids (9). Nevertheless, this occurs mainly in products in which apathogenic staphylococci are used without lactobacilli (18). In traditionally manufactured molded dry sausage (e.g., genuine Hungarian salami), no starter culture and very small amounts of carbohydrate are added; as a result, pH in the final product is around 6.0 (7).

As for breakdown of muscle proteins, endoenzymes (cathepsin D) are active at the beginning of ripening (13) and are enhanced by lower pH (17). Lower pH, on the other hand, does not favor endogenic aminopeptidases. Microbial aminopeptidases that break down peptides into amino acids also contribute to aroma development (19). Their activity is influenced by a_w, pH, and temperature, too. Bacterial enzymes play a role in proteolysis at a later period of ripening (20).

Of microorganisms, the proteolytic activity of lactobacilli, micrococci, and molds has been more thoroughly investigated, and it has been found that some lactobacilli (*L. casei* and *L. plantarum*) can break down sarcoplasmic and myofibrillar protein (21). Intensity of protein metabolism is higher by micrococci (staphylococci) that are also responsible for formation of volatile and nonvolatile aroma compounds (22,23).

Nielsen and Coban (24) found that *Penicillium nalgiovense* has proteolytic activity, breaking down peptides in addition to muscle protein stimulated by salt. Ordóñez et al. (25) found strong proteolytic (and lipolytic) activity with *P. aurantiogriseum*.

In lipolysis, endo- and exoenzymes participate, yet microbial enzymes also play a more intensive role here (26). Typical flavors and aromas are related to the hydrolitic and oxidative changes occurring in the lipid fraction during ripening. Lipid oxidation may cause off-flavor but may contribute to the development of desirable flavor, too (27). Lipid oxidation is mostly associated with unsaturated fatty acids and is often autocatalytic. For this reason, risk is higher if the raw material contains a higher ratio of unsaturated fatty acid (8,28) either by partially replacing fat with vegetable oil or by using the fat of animals that consumed elevated levels of unsaturated fatty acid. Risk of oxidation, on the other hand, can be reduced by smoking and by mold ripening because of the light protection and direct oxygen consumption provided by mold, as pointed out by Ordóñez et al. (25).

It is interesting to note that as a result of microbial lipolysis in mold-ripened Hungarian salami, a significant increase in the acid number of the fat fraction takes place, reaching a value of 15 without any sign of rancidity as judged by peroxide or TBA number or organoleptically (29). Such a high acid number can be detected only in strongly rancid lard with high peroxide number, where because of high temperature rendering no microbial activity takes place and moisture is also very low.

Because physical, chemical, biochemical, and microbiological changes in mold-fermented sausages are rather similar to that of dry sausages without mold, the reader is referred to publications on these topics (2,14,17,19,22,23,27,30–32); mainly those features have been discussed that are characteristic to mold-ripened sausages. It has to be mentioned that sensory characteristics may significantly differ depending on the diameter for two

reasons: the metabolic pathway is different in the case of anaerobiosis (large-diameter salamis) compared to small-diameter sausages, in which anaerobiosis occurs to a lesser extent; a thinner sausage has much less time for aroma formation because of the significantly faster drying.

V. SAFETY OF MOLD-RIPENED SAUSAGES

A. Bacterial Risk

The problem of possible growth, survival, and/or toxin production of salmonellae, EHEC, toxinogenic clostridia, staphylococci, listeriae, and mycotoxic molds has to be dealt with, as well as the potential risk of biogenic amines.

Safety requirements can only be met if hygienically produced raw materials, ingredients and additives are used during good manufacturing practice (GMP), applying a well-based HACCP system. For traditional technologies, long-existing safety records prove the right way of manufacturing (33). Should a new manufacturing technology be started, challenge tests with pathogens must be used. (With enterohaemorrhagic *E. coli* the FSIS requires the following challenge test with raw fermented sausages: it has to be proved that a 5 logarithmic reduction is caused by the given technology.)

It is relatively easy to inhibit growth of salmonellae and clostridia because both are sensitive to low pH and to low a_w, and clostridia are inhibited by nitrite, too. Staphylococci are inhibited by low temperature and low pH, and although they are rather resistant to low a_w, no enterotoxin can be produced at an a_w value that is common with long-ripened sausages ($a_w < 0.90$).

Listeriae are rather resistant to the pH and a_w values of fermented sausages, but if initial count is low and GMP is applied, listeriae are affected by low pH, low a_w, growth, or presence of starter cultures causing reduction in their number mainly if bacteriocinogenic microbes are applied (10,11).

Enterohemorrhagic *E. coli* are also rather resistant to low pH and low water activity, but they are reduced in number if a low a_w (≤ 0.91) condition exists for a longer time at elevated ambient temperature (34). This phenomenon, also called metabolic exhaustion, is a reliable means that helps ensure a five-log reduction in case of long-ripened dry sausages.

B. Mycotoxic Molds

Since the discovery of aflatoxin, numerous other mycotoxins have been detected and identified on agricultural products, in foods, and in nature. Ever since we became aware of the potential risk of mycotoxins, foods with mold growing inside or outside, spontaneously or intentionally, are considered "suspicious." With this in mind, mold-ripened sausages (and hams) have been investigated thoroughly, and the isolated molds were tested for toxin production (35–37). It was possible to isolate mycotoxic molds from mold-fermented meat products, and mycotoxins were also found in products artificially inoculated with pure culture of toxic molds, but in general no mycotoxins were detected in commercially manufactured meat products. In further experiments, the effect of extrinsic and intrinsic factors on toxin production potential were investigated (38,39). In these latter experiments, mixed culture ("house flora") of apathogenic molds, low temperature (13°C), and low a_w-value (0.94) as well as intensive smoking have been shown to be growth inhibitors of mycotoxic molds (8 aflatoxin and 11 sterigmatocystin producers were inoculated). This finding has been supported by investigation of 800 salami samples taken from

retail shops: testing analytically as well as biologically for mycotoxins gave proof of the absence of mycotoxins (40). On the basis of these results, "house mycoflora" of ripening rooms can be considered harmless if traditional technologies with reliable hurdles are applied during ripening-drying; nevertheless, technologies have been changed in general for application of mold starters.

Summing up, we can conclude that fermented, dried meat products are stable and safe without refrigeration because of low a_w-value (< 0.90) or because of a combination of low a_w and low pH value.

C. Biogenic Amines

These compounds are mainly formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketons (41). Occurrence and concentration of biogenic amines (tyramine, putrescine, cadaverine, etc.) in fermented foods have been intensively investigated (42), and it has been found that starter cultures may play a role in the extent of biogenic amine formation but freshness of raw material can be considered a major factor in keeping occurrence and concentration of biogenic amines low (43,44). Consequently, dry sausages properly manufactured from fresh raw materials can be considered safe also from this point of view.

VI. SHELF LIFE

Long-ripened dry sausages are stable from the microbiological point of view because of their low water activity. Nevertheless, if further drying and influence of oxygen are not inhibited, sensory quality is lowered. Vacuum or modified atmosphere packaging (MAP) with high barrier films could extend the shelf life because moisture does not evaporate, and in the absence of oxygen, rancidity is significantly retarded. Although vacuum packaging or MAP is a perfect solution for extending the shelf life of dry sausages without mold, mold-ripened sausages cannot be vacuum packaged, and MAP is not an ideal choice either for longer storage. The problem in both cases is that moisture cannot evaporate from the surface, making it wet; thus, mold loosens and comes off, giving the product a bad appearance. For shorter storage, either wrapping in cellophane or perforated polypropylene (in order that moisture evaporates) can be used, with awareness that weight loss may occur even if reduced; or MAP may be used, in which case weight loss is avoided but saturated humidity does not favor the mold layer, as mentioned above. For this reason, mold-ripened sausages are more affected by storage conditions than sausages that can be packaged in impermeable films; yet mold layer protecting against moisture loss and oxidation (light) ensures a long shelf life for these high-value meat products, supported also by the advantageous effect of smoking, even if they are packaged in moisture-permeable films. Evidently if dry sausage is sliced after peeling off, it can be either vacuum packaged or MAP applied and a long shelf life can be guaranted. Because of less residual oxygen in MAP, less oxydation effect was detected than in vacuum packaging (45).

As already mentioned, long-ripened dry sausages are stable and safe without refrigeration, and cold storage is rather disadvantageous because phosphate crystals can appear on the product. This can be the case even if no phosphate was added: muscle tissues' own phosphate is sufficient for causing this phenomenon, supported by decreasing solubility of phosphate at low a_w and higher pH.

VII. QUALITY DEFECTS

The background of quality defects can be found in use of poor raw materials, in technological failures, and in storage abuses. Some of the more common faults are discussed below.

A. Color Defects

Color defects can be caused by insufficient amount or absence of curing salt by mistake, but mostly by smoking or drying failure. If sausages are smoked with wet surfaces or if surfaces are wet for long, brownish-grayish discoloration occurs, which is most of the time irreversible.

B. Odor Defects

Odor defects can be caused if surface is wet for long, and yeasts as well as micrococci grow, making the surface slimy and malodorous. This slimy layer inhibits good adhesion of molds which can slip off easily. If raw material is not of good hygienic quality or ripening-drying is not done properly (too high temperature, too slow drying, inefficient starter culture, etc.) spoilage can occur with unpleasant odor and taste; health risk cannot be excluded either.

C. Flavor Defects

Flavor defects can be caused if product is too acidic (higher carbohydrate concentration, higher temperature for long) or rancid. This latter can occur if raw material was not fresh, if fat ingredients contain too high concentration of unsaturated fatty acids, drying and storage is done at too high temperatures and too long, no nitrite is used, and sausages are not smoked.

D. Defects in Appearance

A properly manufactured mold-ripened dry sausage has an even mold layer, the sausage is without deformation, and slices are uniform in color and texture. The reason for uneven mold layers may be technological in nature (temperature and relative humidity unfavorable, inefficient mold starter), but in some cases molds with high cellulase activity decompose the cellulose casing and small parts of the casing come off with the molds.

Deformation is caused by inadequate drying (too rapid, mainly in the first period), resulting in case hardening that does not allow steady drying. Case hardening is not only a quality defect: as a consequence, spoilage and/or pathogenic microbes may grow because a_w-reduction is slowed down significantly.

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Meat Products Processing: Operational Procedures and Equipment

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I. INTRODUCTION

Numerous historical documents testify to the fact that many meat products submitted to processes of salting, drying, and ripening may be defined as being traditionally prepared, essentially in accordance with techniques adopted by small family enterprises in former centuries.

On the basis of the modality of preparation and of the processing mechanisms used, products can be divided into two main categories: those based on whole anatomic fractions and the based on minced meat.

II. WHOLE PRODUCTS

A detailed analysis of all whole-fraction-based products prepared in the various regions of the world would be decidedly complex and possibly superficial; it is more useful to select a few products that are representative of the wider spectrum, highlighting their special features and drawing conclusions that can be easily adapted to the others.

A. Production Techniques

Traditional techniques include some simple operations consisting essentially of the preparation of anatomic fractions, the addition of sodium chloride mixtures and of varying ingredients, and the maturation of the product for varying lengths of time in appropriate environmental conditions, the latter being traditionally influenced by climate. In any case, the start of production coincided with the coldest season of the year.

Many present-day techniques are the same as traditional ones, and the optimal thermohygrometric conditions are obtained utilizing appropriate air conditioning systems.

This chapter addresses the problems linked to the preparation of ham, which is considered one of the most important examples, and describes the most significant differences between this product and others. Table 1 is a general scheme of the preparation of cured ham.

Table 1 Preparation Techniques for Raw Cured Ham

Stage	Purpose	Operating conditions
Preparation of the leg	To prepare products for salting	The elimination of part of the rind, fat, and bones
Adding salt	To add salt and other ingredients	The use of salt in the dry state
Cold storage: salting and rest	To allow for the absorption of the salt and for the water loss necessary to reduce A_w	Storage in T- and RH[a]-controlled rooms
Washing	To remove salt residues and to clean the surfaces	Washing by tepid water
Drying/smoking	To dehydrate the external fractions/permit the absoption of some components of the smoke	Storage in T- and RH-controlled rooms/use of product smoking from various sources
Ripening	To facilitate the enzymatic processes responsible for maturing	Storage in T- and RH-controlled rooms

[a] T = temperature; RH = relative humidity.

1. Preparation of the Leg

The salting of the whole pork leg is a practice traditional to many regions, although the forms of preparation (the removal of differing quantities of rind and fat, and of varying parts of the pelvic bone) vary greatly from region to region (1). Besides being linked to traditional practices and food habits, these differing types of preparation reflected the necessity to optimize the main processes: the diffusion of salt and water, and the absorption of the components of the smoke.

A lesser covering of fat and rind, facilitating the processes of diffusion, reduces the probability of microbial contamination and slows down many enzymatic processes that on the basis of recent studies, can lead to the formation of undesirable sensory properties if they are too accentuated (2–4).

It is interesting to note that, in general, the forms characterized by a greater muscle surface freed of fat and rind pertain to regions where the climatic conditions slowed down salt diffusion (cold climates) or where there was a greater probability of undesirable microbial growth (hot climates). The rapid reduction in water activity (A_w) was, in fact, practically the only way to prevent undesirable microbial growth.

2. Adding Salt

Salt is normally added in the dry state or mixed with limited quantities of water. The techniques of salting products vary: in most cases, the entire mass of the product is covered in salt, that in some important techniques (e.g., for Parma ham,) the salt covering is limited to the area where there is the greatest probability of microbial growth (the main blood vessels, areas in proximity to the head of the femur, etc.).

Traditionally, the addition of salt was by hand; nowadays, salting machines are being used more and more, because they allow for the complete or partial covering of the muscle surface without the exertion of mechanical actions that could damage the conformation of the product and/or cause breakage of bones or lacerations of the muscle fractions. The use of tumblers, which may give rise to the above-mentioned defects, is limited to certain production but is not normal practice for the traditional production methods of some countries (Italy and Spain).

Similar situations are encountered in products other than ham; for these, because of the differing conformation and the absence of bones, the use of tumblers is widespread.

3. Salting

a. Hams The salted hams are placed in suitable rooms (salting rooms) to allow for the diffusion of salt in conditions (low temperature) that prevent anomalous microbial growth.

The forming of a saturated solution on the surface of hams is an indispensable condition for the correct diffusion of salt to the more internal fractions (5). The thermohygrometric parameter values should be chosen on the basis of sure scientific ground (e.g. minimum growth temperature, in the pH, and A_w conditions, of the internal fractions, of a reference pathogenic microbe (e.g., type B *Cl.botulinum*). The technologies currently employed are essentially different on account of the thermohygrometric values (temperature and relative humidity) and the modality of storage of the products in the salting rooms.

The salted hams are placed in open containers of varying dimensions, arranged in heaps completely covered in salt or placed individually on shelving or similar supports; the latter technique is the only one adopted for hams partially covered in salt (1,6,7). In the first two cases, only one small fraction of the hams is in direct contact with the air, and the diffusion of the salt and water essentially depends on the characteristics of the salt and ham and on the temperature. In the latter case, these processes also depend on relative Humidity; values near to those of the A_w of the saturated salt solution ($A_w = 0.75$) ensure a good outcome of the process; the optima choice of the control parameters depends in any case on the type of product and conditioning plant.

In many technologies, the salting stage is subdivided into two or more substages. At the end of every substage, the residual salt can be removed and replaced with clean salt, and the hams can be submitted to mechanical massage (manually or with the aid of appropriate machines) to facilitate the removal of any blood still present in the main blood vessels.

The overall duration of the salting stage depends directly on the quantity of salt desired in the finished product and on the diffusion rate, ranging from a few days (for small products completely covered in salt) to several weeks (Parma ham). The differences that are noted in the more traditional products (jamon iberico, Parma ham), and that have similar quantities of salt in the finished product, depend on the differing quantities added at the salting stage, the different modalities of trimming, and the thermohygrometric conditions (8).

Independent of relative humidity values, the preestablished temperatures take into account the need to control microbial growth and to ensure the appropriate diffusion of salt and water, varying within wide limits—for example 36–40°F (9), 1–3°C (10), 2–3°C (2), 0.5–4.0°C (11).

b. Other Products For other products, similar storage techniques are used. If the products are left in tumblers, or are deposited in impermeable containers, the salt absorbs water from the surface fractions and forms a saturated brine; the quantity of brine depends directly on the quantity of salt and of lean meat in contact with the salt; generally, it is not sufficient to cover completely all the product. This may give rise to an uneven production, rectifiable by means of operations (tumbling, changing the loading of the product into the containers, etc.) that allow for a more uniform treatment.

The salting time depends on the quantity of salt and other additives or ingredients to be absorbed (the various ingredients and additives have varying diffusion coefficients) (12); because the processes are slow, the salting period may also be long (e.g., 3–4 weeks for beef products with a heavy weight). During salting, the quantity and concentration of brine diminish with the passing of the days, and the A_w and surface microbial population increase.

c. Brining The addition of salt may also be by immersion or by the injection of brines of different concentrations; in this second case, the microbiological and physicochemical conditions of the internal fractions are also modified to a significant degree, and the salting techniques must also be modified in order to optimize the growth of the characteristic microorganisms present and/or starter cultures (13).

d. Desalting In some countries, prolonged salting is followed by a desalting stage, during which the products are placed in water, sometimes running, for a certain period (more or less one day).

This technique speeds up the salt diffusion by increasing the concentration of the external fractions, and facilitates quality control of the finished product, eliminating a part of the salt absorbed. The necessity to purify large volumes of contaminated water and an increase in the quantity of moisture in the surface fractions are the most obvious limitations of this technique, which in many countries is not accepted.

4. Resting

a. Hams At the end of the salting stage, the A_w of the internal fractions is still very high ($\geq$0.96) (5,14), and hams cannot yet be submitted to the temperature conditions that would facilitate the enzymatic processes responsible for the formation of their fundamental sensory properties.

The hams are thus placed in good drying conditions at refrigeration temperatures; this stage, normally referred to as resting, has the purpose of preventing the growth of and/or inactivating the harmful microorganisms present on the surface, and of progressively reducing the internal A_w to values that allow for the transfer of the product to rooms at a higher temperature.

At this stage (adopted in many technologies) the hams are placed in suitable locations (resting cells) so as to facilitate the exchange of matter (the evaporation of water) with the environment.

The thermohygrometric conditions differ greatly according to the characteristics of the product; in all cases, at first a rapid drying of the surface must be achieved (low relative humidity values and adequate ventilation) in refrigeration conditions (temperature similar to that of the salting stage) for a sufficient number of days (7–14); the drying conditions are subsequently slowed down so as not to cause damage to the external fractions (drying rim).

The duration of the resting stage depends on numerous parameters, in particular those which influence the diminution of the internal A_w, which, not being measurable directly, must be estimated by evaluating the quantity of salt and water in the more internal fractions.

The resting period is longer the greater the weight of the ham and the lower the quantities of salt absorbed, and ranges from a few weeks, for small-sized hams containing higher quantities of salt, to several months for sweeter products (e.g., 2–3 months for the bigger-sized Parma hams). For very small and salty products, this stage can be omitted.

b. Other Products What has been said regarding hams also applies to other products. The thermohygrometric conditions must be chosen according to the same rules;

in particular, the greater the dimensions, the lower the quantities of salt required, the lower the temperatures of the environment, and the longer the overall period of the stage.

The introduction of the resting phase for certain products prepared with limited quantities of salt and additives and of a certain dimension (coppa, dried meat from the Grisons, Bauernspeck etc.) reduces the incidence of microbiological contamination and improves some sensory properties (uniformity of color, greater proteolysis control, etc.).

The resting times at this stage are generally modest (1 to 3 weeks), although there are numerous exceptions, in particular for anatomic fractions isolated from pork legs.

If the product contains nitrates, the thermohygrometric conditions must be such as to facilitate a certain transformation of this additive, and thus it is preferable to adopt higher temperatures than those indicated for the salting stages (e.g., 3–6°C rather than 1–4°C). If the A_w at the end of the salting phase is very high, the resting period can be subdivided into two stages, the first at lower temperatures (1–4°C) and the second at higher ones.

Hams are normally hung on special supports (e.g., frames) with string or a similar material tied to the end part of the leg; for other products, differing solutions are available: some products are placed in individual containers of varying material and shape (including natural and artificial casing), in order to obtain a desired shape.

5. Drying and Ripening of Hams

In traditional productions, with the arrival of spring and of the hotter seasons, the temperature of the ripening environment rose, with a consequent speeding up of all processes linked to the formation of the sensory properties and to weight loss.

At the end of the resting phase, the hams can be washed in tepid water and placed at temperatures close to or higher than room temperature in good drying conditions.

The washed hams are submitted to accentuated drying and, in some cases, to smoking. Temperature choice depends on numerous factors, including the presence of additives, and should take into account the A_w values of the internal fractions.

Generally, it is preferable to adopt lower temperatures at the initial stages (except in some cases for the stages of drying/smoking) and to increase the temperature gradually, as the drying process and the diffusion of the salt facilitate the diminution of the internal A_w.

Mean temperatures are higher for products characterized by more marked flavors (e.g., Spanish ham, with values approaching 33–37°C) and lower for hams having a more delicate taste (e.g., Parma ham and San Daniele ham—generally with values around 15–22°C).

An increase in temperature gives rise to an increase in the water diffusion rate, although the mean rate remains, generally, lower than that of evaporation. Also, in this period, excessive drying leads to reduced permeability of the external fractions.

To reduce the evaporation rate, the relative humidity of the room can be increased or the permeability of the muscle external fractions can be diminished by covering them with a mixture of fat, salt, and flour. High relative Humidity values may facilitate the growth of molds and may require the adoption of specific measures (the use of starter cultures, control of the thermohygrometric conditions, etc.) in order to reduce the likelihood of mycotoxin formation and/or the excess of mold spores in the air.

Hams can be partially isolated from the environment by placing them in appropriate containers (paper or cloth bags, etc.); this measure also helps to protect the product from other pests (mites, flies, etc.), an ongoing problem, which nonetheless goes beyond the scope of this chapter (9). It is, however, important to evaluate to what extent these measures influence evaporation speed and hence the drying of the internal fractions.

6. Differing Techniques

As previously stated, ripening temperatures may vary widely, although, as regards traditional technologies, always in the order of those pertaining to the area of production.

Some products are produced at higher drying temperatures (45–48°C as internal temperatures at end of treatment) so as to give rise to variations in the denaturation of the proteins and to facilitate an increased texture of the product (15). In these conditions, the sensory properties of the finished product are still similar to those of a ripened product, although the preparation times, intended as the time needed for the product to acquire a good consistency, are considerably shorter.

The main differences between these products and the traditional ones undoubtedly have to do with the formation of aromas and to the transformations responsible for conservation under the differing conditions of storage. A careful choice of ingredients allows for the conferring of pleasant aromas and tastes, whereas a higher A_w requires controlled storage temperatures. Raw materials, as well as all technological operations, may contribute to contamination of the product and must therefore be chosen with care; in fact, during the drying phase the internal fraction stays for a certain time (as long as several hours) at temperature values close to those that are ideal for the growth of pathogenic bacteria. It is important to adopt procedures that reduce the initial contamination to a minimum (hygiene measures during processing, the choice of anatomic fractions free of main blood vessels, etc.) and to optimize the heating velocity and the inactivation of the surface microbial flora.

B. Conditioning Plants

As was mentioned above, ripened products remain for long periods of time in locations of varying size in suitable relative humidity, temperature, and air velocity conditions; the conditioning plants play a fundamental role and are responsible for the success or failure of many techniques.

1. Salting Plants

The air in the salting rooms must be at refrigeration temperature and, if the hams are arranged on shelvings, must have an relative humidity value between 70% and 80% and a relatively modest speed (0.2–0.4 m/sec).

In order to meet these requirements, many constructors choose static plants, in which the speed and direction of the air flows are controlled by means of the careful arrangement of heat exchangers fed with fluids at differing temperatures (lower than the dew point and at values that maintain the required room temperatures). The curves describing the relative humidity and temperature variations with time may assume differing forms: those shown in Fig. 1 are characteristic of the salting of Italian products. The ascending part of the curve (indicated as breaking time) coincides with the defrosting of the refrigerator batteries and allows for a certain reequilibrium of the various fractions of the ham; during the cooling phase, excessive drying of the saturated saline solution of the surface fractions of the ham may occur.

The desired conditions (formation of the surface-saturated solution, significant water loss, reduction of the probability of surface microbial growth) can be maintained by controlling relative Humidity evolution with time, which must not vary too much from that of the A_w of the saturated salt solution (0.75).

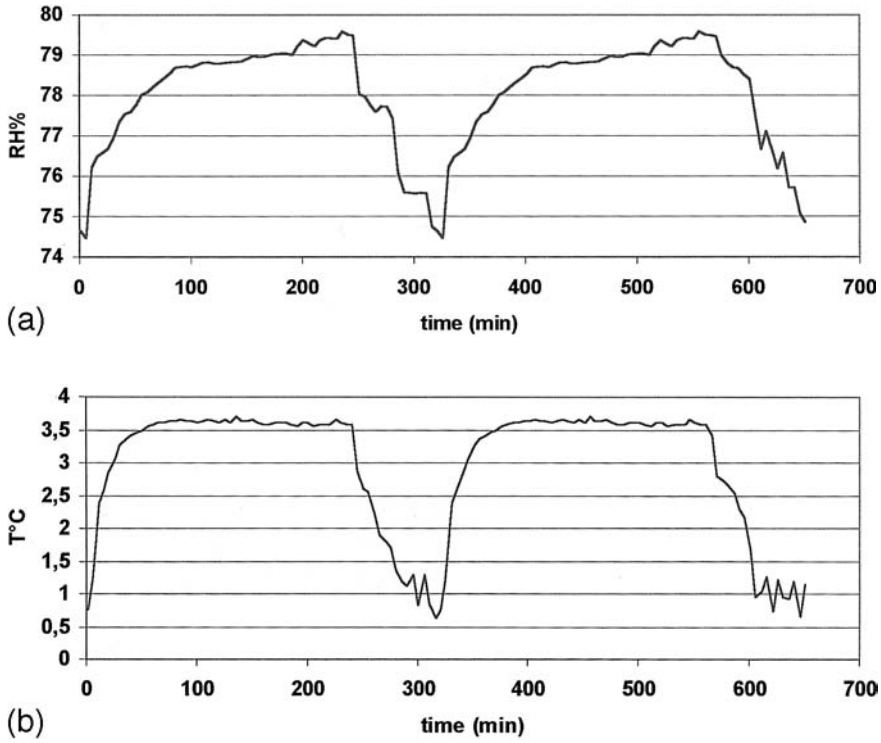

Figure 1 (a) Behavior of RH in salting of Italian hams. (b) Behavior of T in salting of Italian hams.

For other techniques of salting (products in containers with limited contact of the product with the air), the diffusion of the salt and the extraction of water depend essentially on the temperature of the environment, and thus air relative humidity values need not be controlled.

2. Other Stages of Production

The other preparation stages require different thermohygrometric conditions and hence differing conditioning plant characteristics.

In the initial part of the resting phase, accentuated drying is needed (low relative humidity and high air speed) and thus ventilated plants with a high degree of refrigeration power are suitable. The plants utilized for the subsequent resting stages may be the same as those of the initial stage, although the control parameters must be modified—for example there should be an increase in mean relative humidity. These parameters must be chosen on the basis of objective data (temperature, relative humidity, weight loss of the product) and sensory evaluations (hydration conditions of the surface of the product, surface microbial growth, etc.).

During the drying and ripening stages, the temperature of all the heat exchangers can be higher than 0°C; it is thus possible to eliminate the breaking time and to envisage long periods at constant relative humidity equal to a value close to the A_w of the product.

Most plants involve the setting of the minimum and maximum temperature and relative humidity values; in some cases (poor precision and/or uncertainty of measure in operating conditions, cycles characterized by slight relative humidity fluctuations with time) it is advisable to program different control parameters—for example minimum and maximum drying, or cooling, and breaking times. The conditioning plants for products other than ham, including minced meat–based ones, do not differ substantially from those described above.

III. MINCED MEAT PRODUCTS

Products consisting of fractions of lean meat and fat submitted to fermentation processes, drying, and ripening, which will be referred to hereafter by their generic name of salami, are present in many regions of the world; they represent one of the most ancient forms of meat preservation. Characteristics common to all these products are the presence of salt and flavorings and sometimes of other ingredients (sugars) and of additives (nitrates, etc.), and their filling in a casing which is permeable to water vapor, traditionally obtained from animal gut (pig, sheep, horse, cow, etc.).

The compositions and techniques of preparation and ripening differ widely, reflecting the food traditions and climatic conditions of the differing countries of origin. Schematically, the preparation can be subdivided into two main stages: the preparation of the salamis (all the meat fraction cutting operations, the addition of the ingredients, and the filling in varying types of casing), and the drying/ripening phases (all those stages during which the salami is stored in suitable relative humidity and temperature conditions).

A. Main Physicochemical and Microbiological Processes

The physicochemical processes mostly used in the preparation of salamis are the lowering of pH, by the formation of organic acids, and of A_w by evaporation of the water and the consequent increase in the concentration of the various solutes (NaCl in particular).

The curves describing the behaviour of A_w with time are substantially similar in shape (continuous fall during the ripening) for the various types of salamis but differ significantly concerning the values reached, which depend on size, on characteristics (essentially salt water ratio) and on weight loss of the product—for example 0.81–0.87 pepperoni sausages (16), 0.89 salame Milano (17), 0.92 German sausages (18), 0.89 salchichón de cerdo Ibérico (anonymous).

The pH curves present a different behavior: the pH initially decreases until a minimum value and, progresses the ripening, for several products (e.g., Italian and French sausages), tends to rise again. The minimum and final values depend on quantity and type of sugars, on temperature of fermentation, on A_w of product, on type of starter cultures, and on drying and ripening techniques and vary within wide limits (Table 2).

The choice of type of pH and A_w variation with time is one of the most important initial operations in the planning of a technology and/or in the modification of existing techniques; these parameters, in fact, have a decisive influence on the formation of various important sensory properties, including the following:

The texture of the slice
The formation of an oxidation-stable red color (nitrates and nitrites are generally present, whereas colorants are used only in some productions)

Table 2 Minimal and Final pH of Some Types of Salamis

Type of salamis	Reference	Time of phase	pH
German-type salami	13	3 days drying	4.8–5.1
		28 days drying	4.9–5.2
Summer sausages	19	36 hr fermentation	4.85
		60 days drying	5.19
Italian sausage	20	15 days drying	5.45
		45 days ripening	5.7
Salchichon and chorizo	21	2 days fermentation	5.2–5.4
		22 days drying	5.52–5.84

The formation of acid or hot flavors
The formation of specific flavors deriving from the long ripening period and due to the metabolism of the muscle and adipose fractions

Rapid acidification improves the formation of the first two characteristics mentioned above, and ensures a greater inactivation of pathogenic microorganisms, although it increases the possibility of producing salamis with acid and/or hot flavors not acceptable to certain groups of consumers.

B. Technological Operations

Production techniques subsequent to mincing involve a series of specific stages, shown in Table 3. In some techniques, two or more operations are carried out at the same time; only in a few technologies are all the stages effected one by one and separately, being thus easily distinguishable.

1. The Storage of Meats in Refrigeration Rooms

The meat conditioning program must also take into account the subsequent operations; the temperature value must be sufficiently low ($\leq 1^{\circ}C$) for the prevention of undesired microbial growth and for the meat to be minced, mixed, and stuffed in the correct manner. In general, the storage temperature is lower the smaller the particle size after mincing and the higher the quantity of fat in the mixture.

Table 3 Stages in the Preparation of Salamis

Stage	Purpose	Operating conditions
Resting of the meat in a refrigerating room	To prepare the meat for the subsequent operations	$T = 1^{\circ}C$
Mincing and formation of the mixture	To mince the meat, to extract myofibrillary proteins, to add the ingredients	Utilization of the mixture mincing line
Casings filling	To put the mixture into casing	Use of the stuffing line
Ripening	To allow for the formation of characteristic salamis under the effect of microbial and enzymatic processes	Rooms with controlled RH and T

The temperature values normally adopted for the storage of frozen meats (−15 to −20°C) do not seem to be ideal for obtaining regular cuts; the meat should in fact be at temperatures around −8 to −10°C (with a period in a tempering room prior to mincing). These conditions also improve the efficiency of the machinery and/or reduce costs and mincing times.

2. Salami Mincing and Mixing Operations

In order to extract a good quantity of the salt-soluble proteins, the cutting of the various fractions, the addition of ingredients and additives, and the mixing of the various components can be done on one or several machines, each having a specific function.

The most complex line (several machines) involves an initial breaking up of the meat using a roughing machine; the final mincing of the particles takes place in a mincer, and the addition of ingredients and additives in a mixer. This line is not suitable if the quantity of frozen meat is too high in relation to the strength of the mincer or if the particle size is too small and the quality/quantity of fat are such as to give rise to a high possibility of fusion of the adipose fractions. The use of a machine capable of carrying out all the operations required (cutter) entails several advantages, such as the possibility of mincing very hard meat and the reduced likelihood of fusion of the fats, although some problems may arise due to the greater unevenness of the cutting process and the difficulty involved in extracting the proteins completely.

The operating modalities differ according to the type of product desired; the consistency characteristics (a sliceable or spreadable product) must be evaluated.

3. Casings Filling

Stuffing (the placing of the salami mixture into a casing) is in theory a simple operation, although it must be effected with care to avoid the appearance of small cavities and the fusion of part of the adipose fraction.

4. Ripening

During the ripening phase, whereby the product is stored in appropriate thermohygrometric conditions (temperature, relative, humidity), an evolution takes place of the microbiological and enzymatic processes responsible for the preservation of a salami and for the formation of its main sensory properties (18).

In order to optimize the various processes, this stage is normally divided into several substages, characterized by differing values of the thermohygrometric parameters.

As a general rule, five substages can be identified: storage and/or drying at low temperature, heating up to the fermentation temperature, fermentation, drying, and ripening (Table 4).

Only some technologies, those designed to produce salamis with relatively high pH values and long ripening periods, expressly involve the division into all five substages; in many cases, in fact, only the middle stages (heating up to the fermentation temperature, fermentation, and drying) are involved.

The optimization of the various stages requires a knowledge of the evolution of the main physicochemical parameters: temperature, pH, and A_w.

Throughout the ripening period, the latter parameter undergoes a slow diminution directly linked to the humidity loss that takes place in suitable thermohygrometric conditions (relative humidity $< A_w$). The decrease in water content depends on two interdependent

Table 4 Substages of the Aging Process of Salamis

Substage	Purpose	Operating conditions
Resting and/or drying at low T	To allow for an initial drying stage in refrigeration conditions; for salamis at higher pH values	Refrigeration T; RH is chosen on the basis of the casing
Heating to fermentation T	To bring the internal temperature to ideal levels for fermentation	T higher than that of fermentation; RH chosen according to the necessity to avoid water condensation on the casing
Fermentation	To facilitate the metabolism of the characteristic microorganisms, to effect an initial microbial selection	Ideal T for the fermentation of the characteristic microorganisms, RH close to the A_w of the salami
Drying	To effect a good drying of the salami and to start the diffusion of the water from the inside	T and RH chosen on the basis of the techniques adopted (drying at constant or variable RH)
Ripening	To facilitate the formation of the main characteristics of the salami	T and RH chosen on the basis of the internal and surface microbial population

processes: evaporation from the surface and the diffusion of water and solutes (salt, sugars, etc.) in the internal fractions.

The diffusion ratio of the solutes and water depends directly on temperature value and on the characteristics of the product (pH, the A_w of the various fractions, the fat/lean ratio etc.), whereas evaporation is linked to the characteristics of the air (relative humidity, temperature, ventilation) (22) and to the permeability of the casing (11).

The drying of the external fractions determines the diffusion of the salt toward the inside and the migration of the water toward the outside; because the evaporation ratio is generally greater than that of diffusion, a concentration gradient is created and the salami becomes a system consisting of concentric fractions, differing as to quantities of salt and water (23,24).

Excessive drying of the external fractions may give rise to surface proteinic denaturation with aesthetic ("drying rim") or functional damage with reduced permeability of the surface fractions (25).

C. Modality of Drying

Numerous techniques are adopted to control the drying ratio; some require the main thermohygrometric parameters (temperature, relative humidity) of the air to remain constant for sufficiently long times (drying at constant relative humidity levels), since others involve the continuous fluctuation of relative humidity between a minimum and a maximum value (drying at variable relative humidity levels) (Table 5).

In techniques with constant relative humidity, the setting of the relative humidity value equal to the A_w of the product, diminished by a factor linked to the permeability of the casing, allows for constant evaporation. By reducing the difference between relative humidity and A_w, the A_w differences between the various fractions of the product are minimized, but overall weight loss diminishes.

Table 5 Drying and Ripening Techniques

Type of salamis	Reference	Phase	Time	T(°C)	RH(%)
German-type salami	19	Drying	2–4 days	18–25	90–94
		Drying	5–10 days	18–22	80–90
		Ripening	28–56 days	10–15	65–80
Salchichon and chorizo	21	Fermentation	40 hr	27	95
		Drying	21 days	12–15	75–80
Pepperoni	16	Fermentation	1–3 days	35	85
		Drying	40–42 days	12	65
Salame Felino	20	Drying	1st day	20	45–65
		Drying	2nd day	19	55–75
		Drying	3rd day	18	60–80
		Drying	4th day	17	60–80
		Drying	5th day	15	60–80
		Ripening	35–45 days	12–14	75–90

The techniques with variable relative humidity involve more aggressive drying phases regularly interrupted by periods of resting necessary for the characteristics of the external fraction to remain unchanged; during these interruptions, in general the air circulation is stopped, and there is an increase in the environmental relative humidity by evaporation of the water from the product and a rehydration of the external fractions by diffusion of the water from the more internal fractions (Fig. 2).

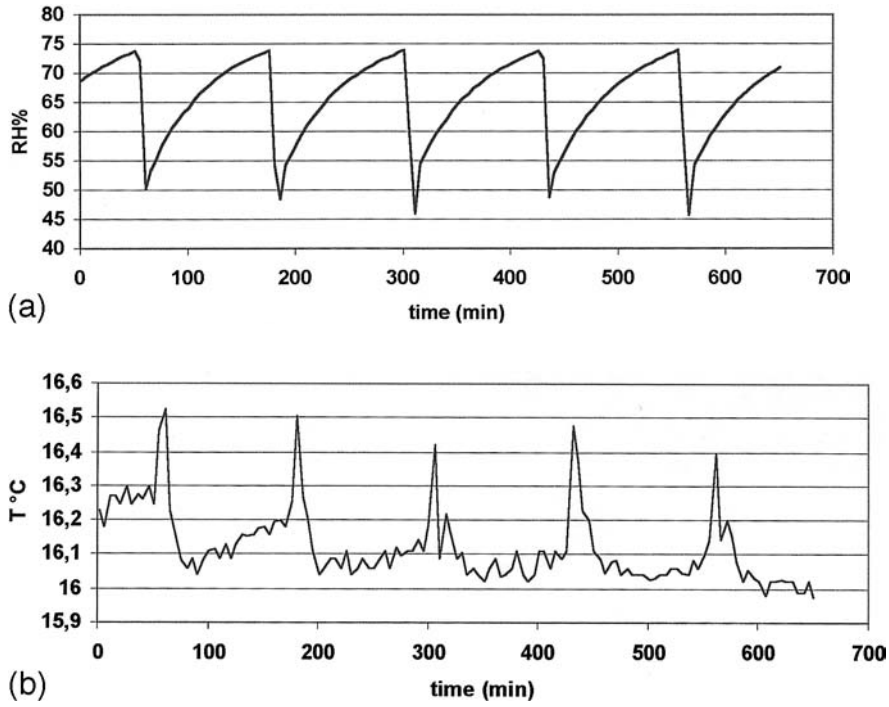

Figure 2 (a) Behavior of RH in drying of Italian salami. (b) Behavior of T in drying of Italian salami.

The profile of the mean relative humidity in the two techniques follows differing trends: ascending in the case of the techniques with variable relative humidity and descending in the other case. The choice of one or the other technique depends on numerous factors, which cannot be more closely examined here. It is important, however, to evaluate the influence of one technique or the other on all the characteristics of the product: internal temperature, surface microbial flora growth, and drying of the casing.

Accentuated drying facilitates a certain diminution, by as much as several degrees in the internal temperatures of the product, as a consequence of the high quantity of heat absorbed by evaporation (on day 2 of drying an Italian salami having a casing diameter = 90 mm, a lean mean/fat = 85/15, the averages of room temperature and internal temperatures were 16.08 and 14.33, respectively; personal data), the selection of the microorganisms most resistant at low A_w values and the rapid drying of the casing.

Traditionally the variable relative humidity technique was chosen for salamis with low acidification and stuffed in natural casing with poor permeability; the second technique was adopted in virtually all other situations.

The values of the main control parameters are defined in differing ways; for the techniques with constant relative humidity values, the choice is based principally on objective parameters (optimal temperature values for the type of fermentation, a preset difference between relative humidity and internal A_w, whereas for those with variable relative humidity values, the experience of the operators counts for more and is helped by the use of systems designed to improve the processes based on statistical techniques. It is not in fact easy to judge solely by means of objective parameters the moment to interrupt drying—that is, when the surface is too dry—and to begin another drying cycle when the surface is sufficiently wet.

Surface microbial flora growth control introduces a further complication to the choice of the relative humidity values: in the case of the constant relative humidity techniques, the choice of the starter cultures is fundamental (26), as is everything linked to environmental pollution. For the other technique, because the salamis are stored for a certain period at relative humidity levels lower than optimal values of the growth of undesired strains, it is possible, by acting on the drying and reequilibrium times, to improve surface microbial flora control.

D. Choice of Temperature and Relative Humidity Values in the Different Periods

1. Salamis with Rapid Acidification

For products with rapid acidification, attention is focused on the fermentation stage, and thus cold drying is not normally present, heating methods are linked to the probability of mold growth in the subsequent stages (relative humidity is controlled to avoid the condensation of water on casing), the fermentation temperature is chosen on the basis of the type of starter culture used (higher for pediococci, lower for lactobacillaceae) (13) and relative humidity is close to the A_w value of the mixture and the time of the stage sufficient to reach a preestablished minimum pH value.

The thermohygrometric parameters (temperature and relative humidity) of the subsequent stages are chosen on the basis of the characteristics of the product during and at the end of the aging stage, because the water retention power diminishes as pH is lowered, and acidic salamis dry more quickly. The control of A_w and of the quantity of water and salt in annulus of the product at the various drying stages allows for the implementation of improvement techniques (22,24).

The sausages can be heated, at the end of fermentation or ripening, to an internal temperature of higher than 60°C to eliminate trichina or Enterobacteriaceae (18).

For salamis, whose preservation depends on a combination of several factors, the main aim of the various substages must be to ensure the inactivation of pathogenic microorganisms without jeopardizing the formation of the traditional sensory properties.

Studies based on "hurdle effect" (18) and on predictive microbiology enable the definition of optimal combinations of the various factors (27,28).

The control of pH decrease is one of the main objectives, and it is made possible by means of a series of considerations based on the treatment of the fresh meat, the choice of ingredients and the ripening techniques.

Because pH decrease is influenced by A_w and the variation in this parameter is closely linked to the diameter of the salami, greater attention is paid to the preparation of bigger-sized salamis.

For these, the technique adopted involves cold-drying for some days (2–3 days at a temperature of 2–5°), humid air heating for the time strictly necessary to bring the internal temperature to values close to those chosen for drying, the fermentation stage, limited to a few hours, drying at not excessively high temperatures (15–25) and ripening, lasting for varying periods depending on the type of product and casing (up to several months), at temperatures around 10–12°C.

During the drying phase, the room temperature shows a downward trend, which is more marked in the first few days, although there are numerous exceptions to this rule, and various methodologies can be identified for salamis having similar characteristics in confirmation of the complexity of the processes involved.

Smoking forms part of the drying and/or fermentation process; to reduce the possibility of the formation of very dark surface colors, it is advisable in certain conditions to submit the salamis to smoking after the external part has reached an acceptable degree of red coloring.

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Fermented and Dry-Cured Meat: Packaging and Quality Control

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I. INTRODUCTION

The packaging of foods is typically designed to maintain the quality of the product throughout its commercial distribution. However, dry and semidry fermented sausages are usually processed once the sausage mix is stuffed into natural or artificial casings. Thus, casings are subjected to heat and drying and have specific requirements such as permeability to moisture and smoke components. At the end of the process, the sausages are sliced and packaged for retailing. This chapter describes the different packaging materials, systems, and applications, as well as current systems and methodologies for an effective quality control of the products.

II. PACKAGING MATERIALS

Packaging is the main preservation technology in the commercialization of food products. Through the correct selection of both materials and packaging technology, food freshness can be maintained to obtain the required shelf life. Traditionally, a package is considered a passive barrier that retards the adverse effect of the environment on the packaged product. The most visual example of such a package is a glass jar. However, packaging science and technology is in constant evolution, and current trends include the development of packaging materials that positively interact with the environment and the food, or even play an active role in the preservation of foods. The use of permeable or perm-selective plastic packages is an example of the former. Finally, active packaging is understood as a packaging system that acts to preserve and improve food quality. This technology makes use of materials that smoothly release antioxidants or fungicides (emitters) into foods or packages or retain oxygen, ethylene, water, cholesterol, lactose, etc. (absorbers) (1).

The packaging of fermented and dry-cured meats is a well-implanted preservation technology, as for most foodstuffs. However, the package does often play an important role in the meat processing, especially in traditional fermented meats. For some products, packaging materials are used to produce the food, as is the case for salami, pepperoni and

other national and local fermented meat products. Once the product is ready for consumption, it is commonly sliced and packaged for retailing.

The variety of packaging materials and structures used to contain these products is so extensive that an exhaustive list is neither feasible nor worthy. It is more convenient to define the diverse possibilities that are commonly applied. First, the natural or artificial materials used for meat production are addressed, then the materials and packaging technologies applied for commercialization.

A. Casings for the Manufacture of Fermented-Meat Products

Sausage-making procedures consist of the stuffing of the mix (meat, fat, starter cultures, and additives) into an elastic tube (casing), which constitutes the skin of the finished product. The characteristics of the tube are highly variable and dependent on the product as well as on the final presentation to the consumer. Many properties of casings such as size, shape, color, printability, and so on can be considered marketing options, but some others should be carefully controlled to obtain the desired product characteristics. The most relevant characteristic of casings for fermented meat products is their permeability to gases (oxygen) and vapors (water, smoke components, etc.). The drying of the product is mostly controlled by water permeating through the casing wall, a mechanism that is also profitable for smoking. Other crucial properties of the skin are (a) the adhesion of the stuffed product to the casing and (b) the elasticity that is basic for the filling process as well as for the shrinkage during product drying.

The most obvious classification of casings is by their nature. Casings can be natural, obtained from biomaterials, or obtained from synthetic polymers. Natural casings are portions of the gastrointestinal tracts of pork, sheep, and beef. At the slaughterhouse, these pieces are cleaned, selected, and stored. The advantages of using natural casings are, among others, good elasticity and tensile strength to facilitate the filling, edibility and easy chewing, good flavor of final product (they do not release off-flavors), and above all, adequate permeability to water and gases (2). Moreover, the sausage shape is variable, resulting in a valuable traditional home-made aspect, although this variation also is often encountered with industrial automation. In general, products manufactured with natural casing are sold as whole pieces.

Artificial casings can be obtained from different sources. Nonedible cellulose-based casings are commonly used in the manufacture of fermented meat products in high-speed industrial lines. Their main characteristics are exceptional uniformity and tensile strength. They are ideal for products that are intended for slicing because they are easily peeled off. This characteristic can be modified by application of diverse coatings; for instance, food-grade protein is used to adhere the casing to the product. Artificial casings present an adequate permeability to water, gases, and smoke; also, the permeation rate through the tube can be adjusted to meet other permeation requirements by perforation (3).

Intermediate between natural casings and paper-derived casings, collagen-derived casings combine the advantages of uniformity and good mechanical resistance with edibility (4). They can be used in industrial stuffing processes as a substitute for cellulose-based materials for dry products because collagen presents enhanced permeability and shrinks with the product. Compared with natural tubes, these casings are not elastic and cannot be overstuffed. Moreover, collagen casings, as a product manufactured from a natural protein, are edible, although they are tough to chew.

For many sausages, the use of plastic tubes is an excellent alternative because they present outstanding shrinkability, processability, and high-barrier properties. However, the latter is a drawback because the release of water through the casing is a must in the

manufacture of the final product. As mentioned for cellulose casings, water barrier can be adjusted by a suitable selection of plastic materials or by perforation.

B. Properties of Packaging Materials

The commercialization and consumer presentation of fermented meat products is highly variable and dependent on the product itself, national or regional market, consumer demands, and so forth. Many products are commercialized in units; that is, they are sold in whole pieces or they are sliced at the grocery according to consumer demand. Most of these products are not closed in a gas-tight package; instead, a net or string is used for hanging. As a consequence of being exposed to air, the products continue changing until the moment of consumption. At present, however, there is an increasing use of modified atmosphere packaging of whole pieces. With this technology, the product quality is maintained more stable during storage and commercialization. Products sliced at the retail level are wrapped with plastic films or plastic-coated paper, or they are packaged in plastic bags. By wrapping, the product is protected only for handling; therefore, the slices must be kept under refrigeration and consumed in a short period. Polyethylenes and flexible polyvinyl chloride are the most popular alternatives for this purpose. Bags can be manufactured with a wide variety of plastic materials. Polyethylene bags closed by a knot, adhesive tape, and so on are the simplest packaging forms. However, some shops have facilities for vacuum packaging. In this case, more complex plastic structures can be used (i.e., polyamide//polyethylene laminates) and the packaged products enjoy an extended shelf life, even at room temperature.

During the last decades of the 20th century, there was a significant change in the distribution channels from small groceries to medium and large supermarkets. This change results in modifications on the type of product presentation. With some exceptions, products are sliced and packaged at the manufacturer plants. Consequently, the packaging materials play an important role in the maintenance of product quality.

Sliced meat products are sold in a wide variety of packages, although they are mostly made of plastic materials. In contrast to glass or metal containers, which present well-defined characteristics, polymers vary widely in their properties. From the hundreds of thousands of different grades of existing polymeric materials, about 30 families are used in the manufacture of packages, and although they may present a very similar visual appearance, their physical, mechanical, thermal, and barrier properties largely vary—from very flexible and tough to rigid and fragile materials (5). Moreover, these materials can be processed with similar technologies, and therefore, combined, blended, or laminated to adjust the final package properties to specific product requirements.

Because barrier properties to gases (oxygen) and vapors (water, smoke, aroma) are crucial in the preservation of product quality, the permeability characteristics of materials and structures are parameters to take into account during packaging design. It is well known that plastic materials allow the passage of small molecular-size substances through the solid matrix. This mass transfer mechanism is responsible for oxygen or water permeability, flavor scalping, and migration of polymer residues or additives. Although all these processes must be known and controlled, the permeability to oxygen and water vapor is especially relevant to the shelf life of packaged foodstuffs.

Unlike glass or metal, plastic materials are not impermeable to the transfer of low-molecular-weight substances and consequently, there will always be an exchange of oxygen or water vapor between the external and internal atmospheres in a plastic packaged product. However, the rate of this exchange may vary up to six orders of magnitude among plastics. The barrier properties of plastics are commonly represented by their permeability coefficients (P). The permeability coefficient of a plastic to a gas is defined as the volume of gas (V,

measured at standard temperature and pressure conditions) passing through a film of thickness L, per unit of surface area (A), time (t) and gas pressure difference between the atmospheres in contact with both sides of the film (ΔP). The permeability to condensable substances (water vapor or aroma components) is expressed in mass of substance (m) instead of volume (6).

$$P = \frac{V \cdot L}{A \cdot t \cdot \Delta P} \qquad \text{or} \qquad P = \frac{m \cdot L}{A \cdot t \cdot \Delta P} \tag{1}$$

Many packaging solutions make use of films coated with other polymers, metals (aluminum), or metal oxides (silicon oxide, SiOx, or aluminum oxide, AlOx). Because these materials are heterogeneous, the use of permeability coefficients as parameters to express their barrier properties is substituted by permeance values ($\wp$) for oxygen or water vapor transmission rates (WVTR), which take into account the total film thickness.

$$\wp = \frac{V}{A \cdot t \cdot \Delta P} \qquad \text{or} \qquad WVTR = \frac{m}{A \cdot t} \tag{2}$$

Table 1 lists the permeability values to oxygen and water vapor of commodity materials commonly used in packaging manufacture, and oxygen permeance and WVTR

Table 1 Permeability to Oxygen and Water Vapor of Common Plastic Materials and Oxygen Permeance and Water Vapor Transmission Rate Values of Complex Structures

Material	Thickness, μm	Water P (or WVTR), 10^{18} Kg.m/(m^2.s.Pa) (or 10^{12} Kg/(m^2.s.Pa))	Oxygen P ($\wp$), 10^{21} m^3.m/(m^2.s.Pa) (or 10^{15} Kg/(m^2.s.Pa))	
			Dry	Wet
PVOH		485000	0.17	900
EVOH		17000	0.77	91
PAN		2420	1.9	
PAN (70%AN)		8250	10.5	31
Homo PVdC		30.53	4.5	
Co PVdC		242	11.1	
PAAr		6060	3.1	31
PA-6		20600	52	225
Amorphous PA		2420	83	60
PET		2300	135	
PP		726	6750	
PC		19400	10500	
LDPE		1200	21500	
LCP		10	0.42	
PET/PVDC	12/5 g/m^2	170 (14)	17.5 (1.4)	
PA/PVdC	25/5 g/m^2	160 (13)	18.2 (0.72)	
PP/PVdC	20/5 g/m^2	43 (3.5)	25 (1.3)	
PET-met	12/OD. = 2.4	58 (4.9)	3.5 (0.32)	
PET/A1Ox/PE	12/0.05/50	21 (1.8)	7 (0.12)	15 (0.23)
PET/SiOx/PE	12/0.05/50	16 (1.3)	4.9 (0.08)	
PA/SiOx/PE	15/0.05/50	32 (2.6)	7.7 (0.12)	
PP/SiOx/PE	20/0.05/50	13 (1.1)	81 (1.2)	

Source: Ref. 6.

of some structures. As can be seen in Table 1, the barrier characteristics vary among materials by several orders of magnitude.

The selection of suitable materials for package design is based on the food product sensitivity to oxygen and water, shelf life needed for commercialization, temperature of storage, as well as mechanical requirements and other properties that are dependent on the packaging technology. As a consequence of the wide variety of these parameters in the case of fermented and cured meats, it is not feasible to provide an exhaustive list of packaging solutions. Instead, it is more practical to describe some typical solutions used with three packaging technologies, more widely used to preserve these products, described later in this chapter.

III. PACKAGING SYSTEMS

After the slicing process, the product is no longer protected by the casing (dry fermented sausages) or by the food manufacturing process (dry-cured ham). The exposure surface of the product to the environment is largely increased and subsequently, rehydration, dehydration, and oxidation significantly decrease shelf life. Plastic packaging becomes essential to delay these processes. In general, the sliced product will exchange water with the environment at a faster rate, resulting in detrimental changes. Similarly, the unfavorable effect of oxygen will be enhanced. Therefore, the requirements for packaging materials and packaging technologies are high water vapor barrier, high barrier to oxygen, and reduced headspace, or headspace composition with low oxygen levels. Among the available packaging technologies, vacuum packaging and modified atmosphere packaging (MAP) are traditionally used for the preservation of sliced fermented and cured meat products. Present and future trends include the use of active and intelligent packaging.

A. Vacuum Packaging

Perhaps the most simple solution to limit or delay the adverse effect of oxygen in food products is the use of vacuum packaging. This technology is based on the reduction of the oxygen available in the headspace of the package by decreasing the headspace volume and/or total pressure. Although some authors include vacuum packaging among the MAP technologies, the headspace composition in the package is identical to that of the external environment.

In vacuum packaging, the food is introduced in a flexible plastic pouch, which is sealed in a vacuum thermosealing machine. The pouch is loaded in the vacuum chamber where air is evacuated, then the open end of the pouch is sealed, and finally, the chamber is vented to atmosphere. The external pressure drives to a collapse of the package onto the product (see diagram in Fig. 1) (7).

The materials used in the design of these pouches should provide a high barrier to gases in order to maintain vacuum during shelf life. Besides this obvious requirement, the materials must be flexible enough to take the shape of the contained product, and present good puncture resistance. Among plastic materials, and suitable for vacuum technology meeting these requirements, is the polyamide family (PA), especially polyamide 6. Unfortunately, these polymers cannot produce a gas-tight seal by application of heat and pressure. Therefore, PA is commonly laminated with polyolefins (low density polyethylene [LDPE], linear low density polyethylene [LLDPE], etc.). In PA/PE structures, the PA provides a barrier to gases and mechanical strength and the PE provides sealing capacity and improved water barrier.

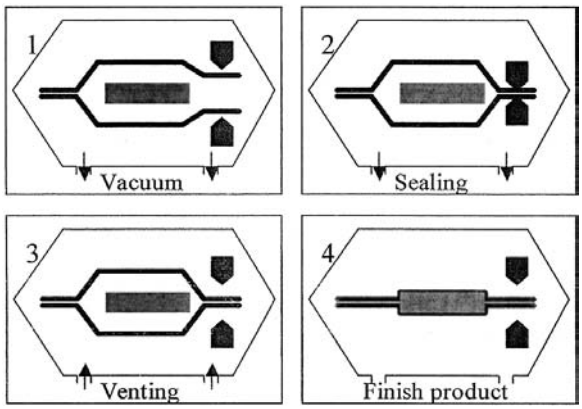

Figure 1 Operation scheme of a chamber vacuumizing machine used in vacuum packaging with a flexible pouch.

The use of vacuum packaging also requires good resistance of the food product to package collapse and to the external pressure. To avoid the adhesion of the slices, pieces of plastic film are used as slice separators. When the product cannot withstand this pressure, a rigid thermoformed tray can be used as container. Some of the structures used in the manufacture or these trays are polystyrene/ethylene-vinyl alcohol copolymers/PE (PS/EVOH/PE), polypropylene PP/EVOH/PE, PA/EVOH/PE, or PET/EVOH/PE (8). These trays must be sealed with a film structure with high enough tensile strength to avoid packaging collapse. Bioriented films containing PET or PA are commonly used for this purpose.

Depending on the required shelf life and storage temperature, the level of barrier needed varies a great deal. Products with extended shelf life stored at room temperature require materials with superior oxygen barrier. Some designs include ethylene-vinyl alcohol copolymers, EVOH, such as PA/EVOH/PA/PE, films coated with polyvinyledene chloride such as PA/PVdC//PE, or coated with metal oxides such as PA/SiOx//PE, PET/AlOx//PE, or PET/SiOx//PE (8). Short shelf life and refrigerated temperatures permit a reduction of barrier characteristics and even single-layer HDPE may be an adequate solution.

B. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is a technology in which headspace air is replaced by a gas mixture that improves and extends the preservation of the food product. There are two basic procedures: application of vacuum and venting with the gas mixture, or flushing the container with the modified atmosphere. The first procedure results in a very efficient replacement of air. The final headspace composition is practically identical to the gas mixture; the gas consumption is low but the packaging process time is longer. The flushing procedure always leaves a residual presence of air and increases the consumption of gas, although the packaging speed is much faster.

Modified atmosphere packaging is applied to many food products, red and white meats, fish, fresh produce, bakery products, and so on. The gas mixture composition varies with the product. For fermented and cured meat products, it is critical to reduce the presence of oxygen. Most solutions are based on the replacement of atmospheric air by carbon dioxide (20–30% carbon dioxide and 80–70% nitrogen). These atmospheres reduce fat

oxidation and inhibit the growth of microorganisms (high levels of carbon dioxide have bacteriostatic and fungistatic effects) (9). Besides maintaining the quality of the product, MAP does not lead to package collapse; consequently, there is no adhesion between slices, and film separators are no longer needed.

The type of container used in MAP ranges from flexible pouches to semirigid or rigid trays. Flexible pouches are the simplest low-cost packaging technology and can be used in fast flow-pack machine with gas replacement by the flushing procedure. However, adequate information must be provided to the consumer. In many countries, consumers are used to buying fermented and cured meats in vacuum packaging. The presence of MAP pouches on shelves is interpreted by consumers as pouches that have lost the vacuum and thus are rejected. Also, the final presentation of the product differs among different pouches, becoming unappealing to the consumer. Therefore, the application of MAP for meat products is commonly carried out in semirigid and rigid trays and the gas replacement is obtained by the vacuum procedure. To better maintain the integrity of the package, total pressure inside the package is maintained slightly below 1 atm.

With respect to packaging material requirements, the properties of the package are similar to those described in the vacuum technology section. Therefore, packaging solutions are similar to those presented there.

C. Active Packaging

Present and future trends in packaging technology include the development of packages that actively interact with the product or headspace, improving and maintaining the quality of the packaged product. These packaging systems are known as active packaging. Materials with oxygen, carbon dioxide, ethanol or ethylene scavenging or generation characteristics are already in commercial use (1).

For products sensitive to oxygen, such as fermented and cured meat products, the use of materials with oxygen-scavenging characteristics results in an extended shelf life. The scavengers decrease the oxygen initially available in the headspace and retain oxygen passing through the package walls due to permeation processes. There are two procedures for the manufacture of a package with scavenging properties. Traditionally, the scavenger was introduced in the package by means of a small pouch manufactured with an unbreakable material (such as Tyvek). However, this procedure is being questioned because this pouch, containing substances that may be toxic, is in direct contact with the food product. Currently, materials are being developed in which scavengers are introduced within the walls of the package, providing similar scavenging properties while being unnoticed by the consumer. The scavenger may be active from the moment it is manufactured but it can also be prepared in such a way as to act at a later stage (at the packaging line) (10).

IV. QUALITY DEFINITION AND DESCRIPTION

The concept of quality may have large variations, but essentially it represents those characteristics of a food that satisfy the demands of the consumer in terms of safety, nutritional value, and sensory characteristics (11). There are many factors (raw materials, additives, conditions of fermentation, drying and ripening, etc.) during the processing of meat products that may introduce high variability into the final quality. Thus, in order to standardize the quality of these products, it is extremely important to have available techniques, such as those described below, to measure quality characteristics, which will

be decisive for consumer acceptability. This is especially important in the case of fermented sausages and dry-cured ham because retail shoppers evaluate the size, shape, distribution of lean and fat, color, and general appearance of the product shown on retail display.

V. QUALITY CONTROLS

Quality constitutes a key factor in commercial transactions in the global world market and within each particular country. Meat processing industries need control systems to guarantee their products and comply with market demands and legislative requirements.

A. Control of Physical Characteristics

1. Drying

There is a trend, closely related to the thermohygrometric conditions of the air (temperature, relative humidity, and air rate), toward variations in water quantity between external and internal areas of the sausage (12). Thus, sausages of larger diameter must be carefully controlled during drying, as the equilibrium between diffusion of the water through the sausage and evaporation rate from the outer sausage is difficult to reach. If drying is too fast (i.e., high temperature or low relative humidity in the chamber), an excess of moisture is removed by evaporation from the outer part of the sausage. This can be visually observed in the cross-section by color fading in the center of the sausage (pinkish due to high moisture content) and a darker color in the outside surface (dark red due to low moisture content). Hardening in the external part is an associated phenomenon.

Water content of sausages and hams constitutes a good indication of the length of the process. Thus, fully dried sausages may reach 20% water losses and a water activity around 0.90. Some Mediterranean sausages are further dried to water losses below 20% and water activity below 0.82 (13). Dry-cured ham reaches about 30–34% of weight loss and water activity below 0.88 (14).

2. Texture

Consistency is related to pH and water loss (15). Some typical defects are related to defective drying processes. When the sausages are of small diameter, it is usual to find a hard texture due to an excessive drying as a result of the use of high temperature, low relative humidity, and/or high air rate. These processing parameters must be controlled because consumers tend to reject tough sausages. When the sausages have larger diameters, a hard dry area on the outer sausage surface may be formed under similar circumstances and act as a shield, retaining most of the moisture and leaving a soft area inside the sausage. This is known as case hardening.

Texture measurements in the form of texture profile analysis may be performed, obtaining parameters such as hardness, springiness, cohesiveness, adhesiveness, and chewiness (16). Another type of test may consist of the assessment of resistance to penetration as an index of hardness during mastication (17).

An excessive softness inside the sausage may be due to different causes (14): (a) the application of nonefficient drying (i.e., high relative humidity) that results in poor water loss and an excess of moisture inside the sausage, (b) fat smearing that retains water, (c) insufficient pH drop, (d) an excess of fat in the mix, or (e) a very fast drying (high temperature, low relative humidity, and high air velocity) that produces hardening of the outer sausage surface but retains most of the moisture inside the sausage. Fermentation (correct sugar

addition, temperature, and starter cultures function) and drying, especially relative humidity and air rate within the chamber, must be carefully controlled.

3. Color

The assessment of color is a useful tool to measure and quantify production and processing treatments. Color may be influenced by pH drop rate and the ultimate pH as well by the presence of other ingredients like spices (i.e., red pepper). Myoglobin is the major pigment in meat, from 50% to 80% of the total pigment (18). Myoglobin content varies depending on the physiological role of the muscle but, as animals grow older, the myoglobin content increases within the muscle (19). This is one of the reasons why older pigs are preferred (better red color than meat from younger animals).

When nitrate is added to the mix in certain long-processing dry fermented sausages, some time must be allowed before pH drop for the action of nitrate-reductase. This enzyme, present in Micrococacceae, is inhibited at acid pH, being then unable to reduce the nitrate to nitrite (20). One of the consequences of this is the lack of adequate nitrosomyoglobin formation and color development (21). Lack of correct color development during the processing may be the result of unappropriate meats. So, raw materials must be controlled for pale, soft, and exudative (PSE) pork meats, which have a trend toward paler color, or meats with low pigment content, which gives poor color and is the case of meats from too young pigs, as mentioned above. Color may also be deteriorated by an excessive acid production or the development of greening by certain strains of lactobacilli or streptococci (15).

Color measurements are convenient to take just after cutting the samples in order to avoid color degradation by either light and/or oxygen. The CIE*L*a*b color space and the Hunter Lab system make use of two types of illuminants, D65 and C, respectively, that are the most frequently used for lighting condition standardization (22). They allow the determination of the color coordinate L (lightness) and chromatic coordinates a (red-green) and b (yellow-blue). Recommended standard observer visual angle is 10° (23). Calibration must be performed using a black standard as $L = 0$ and a white standard as $L = 100$. It is better to homogenize the sample instead of using the full cut surface, due to large variability in measurements as a result of variations in the lean-to-fat ratios in the path of the incident beam (24). Lightness (L) has been reported to increase during the initial stages of ripening. Yellowness (b coordinate) slightly decreases during the full process and redness (a coordinate) increases during the fermentation (25).

B. Control of Chemical Characteristics

1. Composition

Moisture, protein, total fat, and salt are usually determined according to AOAC methods (26). pH is also determined in order to control the extent of acidification during fermentation and later changes, usually by a slight increase, during drying/ripening. Water activity is used in Europe for product standardization. (27). The ratios of moisture to protein and salt to moisture are very useful parameters for controlling the extent of drying and the diffusion of salt inside the sausage (28). In fact, the moisture/protein ratios are required by USDA for dry and semidry U.S. products (see Chapter 22).

2. Biochemical Markers

Several amino acids, D-alanine, D-aspartic acid, and D-glutamic acid, have been proposed as markers of the fermentation and ripening processes (29). These amino acids have also

been monitored during the processing of salamis, and it has been confirmed that the enantiomeric ratio of D and L amino acids correlated with the ripening process (12). D-amino acids are formed through microbial metabolism and L-amino acids through enzyme proteolysis; therefore, these ratios could give a good indication of the balance between both and, thus, for the assessment of the ripening status. The lipolysis and the resulting release of free fatty acids are also the result of the combined action of microbial and endogenous lipases (30,31).

In the case of entire pieces, where time of ripening is essential for correct flavor development, the ratios of certain free amino acids give a good indication of the processing time, being therefore a useful tool for the control of the final quality (32). It is very important to identify the genetic types of the crossbreeds used as raw materials for dry meat products, because they affect its final quality (33). Studies on muscle enzymes and other biochemical compounds performed with different pig cross-breeds (34,35) have found different biochemical profiles in the skeletal muscles, which may have strong importance in long processes such as those used for dry-cured ham or dry fermented sausages with long ripening times. The age of the animal is also very important to the muscle enzymes profile (36). The assay of some of these enzymes has been proposed as a good marker that may help in the prediction of the final quality (37).

3. Flavor

Control of flavor development is very important in order to get final products with optimal quality. In some cases, an excess of certain taste or aroma compounds may impart unpleasant flavors to the product. This is the case with an excess of lactic acid produced during the fermentation stage that results in a sour taste, a defect that is more frequent in sausages with larger diameters. Control must be exerted on (a) the amount of sugars initially added to the mix, because an excess can contribute to a higher lactic acid production and accumulation, (b) the type of sugar—important because glucose or saccharose are easily fermented by lactic acid bacteria, and (c) the temperature for fermentation, because temperatures facilitate lactobacilli growth and more intense lactic acid generation (13). Muscle and microbial aminopeptidases contribute to the final flavor of the product (38). However, an excess of proteolysis may impart a bitter taste to the product. This is the result of an excessive accumulation of free amino acids, such as phenylalanine and tryptophan, with bitter taste (39). This proteolysis may be checked by analyzing the levels of generated free amino acids through phenyl isothiocyanate (PITC) derivatization and separation on reverse-phase HPLC (40–42). The action of the endogenous muscle aminopeptidases is controlled by salt (43) but also by other factors such as certain skeletal peptides (44).

If lard is used as a raw material, its level of unsaturation and the presence of oxidized fatty acids must be controlled in order to avoid unexpected oxidations and thus the development of rancid off-flavors and yellowish color in the fat. This is a usual problem when lards have been stored for long times. It is also important to use starter culture strains with catalase activity and avoid the presence of oxygen during the processing, which is usually accomplished by mixing and stuffing under vacuum. Raw meats must also be controlled for androstenone, the male hormone 5-α-androsten-16-en-3 one having an unpleasant urine-like off-flavor.

Aroma compounds, isolated by dynamic headspace extraction or solid phase microextraction (SPME), are routinely monitored by gas chromatography with flame ionization detector (GC-FID) and/or analyzed by gas chromatography with mass spectrometry (GC-MS) for identification of each volatile compound (45,46). Recent development of electronic noses are promising for effective monitoring of sausage fermentation (47,48).

4. Appearance

Some visual appearance attributes, listed in Table 2, must be controlled in the cross-section of the fermented sausage. They give an approximate idea of the quality of the product. In case one of these attributes fails to appear, the causes must be found by careful screening of the raw materials and right through to the end of the process (see Table 2). Most of the failures are due to inappropriate raw materials or defective processing. Depending on each particular case, control criteria must be adopted as described previously.

In some cases, formation of a white film may be observed on the cut surface of vacuum-packed slices kept under refrigeration. This process is very fast, especially on the surface of slices in contact with the plastic bag. This phenomenon has been typically observed in cut surfaces of dry-cured ham (49) but may also appear in certain dry sausages. The structure of the film has a foam-like fibrous structure (as observed by scanning electron microscopy) and appears to be mainly composed of sarcoplasmic and myofibrillar proteins with low molecular mass. There is also a minor amount (below 12%) of free amino acids, especially tyrosine (50).

C. Control of Microbial Spoilage

1. Off-Odors

Some off-odors may be an indication of undesirable microbiological growth, like the development of putrefactive odors by facultative anaerobic bacteria, usually accompanied by green or gray colors, resulting from muscle pigment degradation and holes or fisures due to gas generation. The usual causes of undesirable microbial growth are related to lack of hygiene and/or incorrect refrigeration during processing, use of contaminated meats or dark, firm, and dry (DFD) meats (with high ultimate pH) prone to contamination, insufficient pH drop during fermentation, or some areas with high moisture content due to incorrect drying.

2. Amines

Biogenic amines may be found in fermented sausages, although in low amounts and with large variability, depending on the type of product and processing. Tyramine is the amine generated at the highest rate (51). These amines may produce migraines or allergy-like symptons in consumers (13). Control must be exerted on microorganisms with decarbox-

Table 2 Appearance Attributes of Fermented Sausages and Possible Sources for Failure

Desirable attribute	Possible sources of failure
Uniform color through whole cut section	Raw materials, excessive drying
Clear-cut section with defined fat globules	Unsaturated fats, cutting, mixing
Homogeneous distribution of fat globules	Cutting, mixing
Correct reddish color in lean	Raw materials, additives, peroxide-forming bacteria
Correct white color in fat	Raw materials, unsaturated fats oxidation
No holes or fisures	Raw materials (contamination), stuffing, heterofermentative bacteria
Casing attached to the sausage	Raw materials (contamination), stuffing, drying
Clean outer surface of the casing	Molds, chemicals
Rigid structure	Low acidification, poor water loss

ylase activity that can generate amines from respective amino acids (i.e., tyramine from tyrosine, phenylethylamine from phenylalanine, etc.).

3. Mycotoxins

The production of mycotoxins depends on the nutritive media, pH, a_w, temperature, and time of storage. Growth of undesirable toxinogenic molds must be prevented in mold-ripened sausages to eliminate the risk of mycotoxin and antibiotic formation (13). The color (green, black, violet, etc.) will also vary depending on the type of mold growing on the surface. Toxinogenic molds may be prevented with antifungal smoke constituents (smoked sausages), growth of suitable nontoxic mold strains (mold-ripened sausages), or dipping in solutions of antifungal agents.

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27
Meat Processing Plant Sanitation

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I. INTRODUCTION

Emphasis placed on food safety has necessitated that rigid hygiene practices be followed in the production and processing of meat, with increased need for workers' understanding of sanitary practices. A major challenge is to protect the production and storage areas against pathogenic microorganisms. Microorganisms are omnipresent and can infect meat, with potentially dangerous consequences to consumers.

Although most plants are hygienically designed, meat can be contaminated with spoilage microorganisms and pathogens. However, safe meats can be produced in older plants if sanitary practices are in place. Hygienic practices are as important to the wholesomeness of meats as are the characteristics of the physical plant.

II. WHY SANITATION?

The following are concrete examples of why rigid sanitation practices are essential:

1. Meat is vulnerable to attack by microorganisms when unsanitary conditions exist.
2. Microorganisms can cause product discoloration, flavor degradation, and illness.
3. The reputation of a firm depends on the safety and quality of its products.
4. Consumers demand and expect to receive safe food.
5. Rigid sanitation and temperature control can improve product shelf life.
6. Food safety emphasis by regulatory agencies has increased.
7. Employees deserve clean and safe working conditions. A clean and uncluttered environment can improve morale, productivity, and employee turnover.

III. WHAT IS SANITATION?

Sanitation is "the creation and maintenance of hygienic and healthful conditions." Meat sanitation is an applied sanitary science related to the hygienic processing, preparation, and handling of meat. Sanitation applications refer to hygienic practices designed to maintain a clean and wholesome environment for meat production, processing, and storage.

IV. ROLE OF MICROORGANISMS IN MEAT SANITATION

Microorganisms (also called microbes and microbial flora) exist throughout the natural environment. They cause food spoilage through color and flavor degradation and food-borne infection if microorganisms of public health concern are ingested. Microorganisms are important in connection with meat sanitation because certain disease-producing microorganisms may be transmitted to foodstuffs. Thus, hygienic practices are needed to combat the proliferation and activity of pathogens and food spoilage microorganisms.

A microorganism is a microscopic form of life found on all nonsterile matter that can be decomposed. Microbes metabolize in a manner similar to humans through nourishment intake, waste products discharge, and reproduction. Improved sanitation is responsible for reduced contamination and increased product stability.

Meat discoloration is associated with the amount of oxygen and carbon dioxide present. High carbon dioxide partial pressure can cause a gray or brownish discoloration by association of carbon dioxide with myoglobin at the free binding site, and the rate of metmyoglobin formation increases with decreasing oxygen pressure.

A principal cause of a discoloration is microorganisms that consume available oxygen required to maintain the muscle pigment myoglobin in the oxymyoglobin state. Oxidation effects are manifested through an abnormal brown, gray, or green discoloration of muscle tissue by oxidation of iron to the ferric state and direct attack by oxygen on the porphyrin ring. As indicated by Marriott (1), the color of fresh meats becomes unacceptable when metmyoglobin approximates 70% of the surface pigment. Formation of metmyoglobin is enhanced by decreased oxygen pressure resulting from oxygen consumption through growth of aerobic microorganisms. Rapid oxidation to metmyoglobin occurs below 4 mm of oxygen.

Bacteria cause meat discoloration through the reduction of oxygen tension. Discoloration is based on (a) oxidation to metmyoglobin at intermediate levels of oxygen demand of the surface tissue, (b) control of pigment oxidation and reduction by adjustment of oxygen level to the storage atmosphere, and (c) inhibition of high oxygen uptake rates and the preservation of color under atmospheric conditions.

V. MICROBIAL CLASSIFICATION

The microorganisms most common to food are bacteria and fungi. The fungi, which are less common than bacteria, consist of two major microorganisms: molds (which are multicellular) and yeasts (which are usually unicellular). Bacteria, which usually grow at the expense of fungi, are unicellular. Viruses, which are transmitted more from person-to-person than via food, can present a problem among unhealthy employees. A brief discussion of these microorganisms will follow.

A. Molds

Molds are multicellular microorganisms (eukaryotic cells) with mycelial (filamentous) formation. They possess tubular cells that range from 30 to 100 μm in diameter, called hyphae, which form a macroscopic mass called a mycelium. These microorganisms are characterized by the display of a variety of colors and are normally recognized by their mildewy or fuzzy, cottonlike appearance. They can develop numerous tiny spores that are found in the air and can be spread by air currents. Molds normally withstand wider

variations in pH than do bacteria and yeasts and can usually tolerate greater temperature variations. Although the optimal pH for mold growth is 7.0, a range from 2.0 to 8.0 is tolerated, though an acid-to-neutral pH is preferred. Molds proliferate more at ambient temperature then in a colder environment, even though growth can occur below 0°C.

Most molds prefer a minimum water activity level of approximately 0.90. However, osmiophilic molds do occur at a water activity as low as 0.60. At a water activity level of 0.90 or higher, bacteria and yeasts grow more effectively and normally utilize available nutrients for growth at the expense of molds, which are more likely to grow when the water activity level goes below 0.90. Most molds do not cause health hazards, but some produce mycotoxins that can be toxic to humans.

Molds cause various degrees of visible deterioration and the decomposition of meat products. Their growth can be identified through the observation of rot spots, scabs, slime, cottony mycelium, or colored sporulating mold. Molds may produce abnormal flavors and odors due to fermentative, lipolytic, and proteolytic changes caused by enzymatic reactions with carbohydrates, fats, and proteins.

Molds have an absolute requirement for oxygen and are inhibited by high levels of carbon dioxide (5–8%). The diversity of molds is evident through their ability to function as oxygen scavengers and to grow at very low levels of oxygen—even in vacuum packages. Some halophilic molds can tolerate a salt concentration of over 20%.

B. Yeasts

Yeasts differ from bacteria in their larger cell sizes and morphology. The generation time for yeasts is less than for bacteria, with a typical time of 2 to 3 hr in foods, leading from an original contamination of one yeast per gram to spoilage in approximately 40–60 hr. As with molds, yeasts can spread via the air. Yeast colonies are normally moist or slimy in appearance and creamy white. Yeasts prefer a water activity level of 0.90 to 0.94, but can grow below this range. In fact, some osmiophilic yeasts can grow at a water activity level as low as 0.60. These microorganisms grow best at a pH of 4.0 to 4.5. Yeasts are more likely to grow meats with a lower pH and on those that are vacuum packaged. Meat products that are highly contaminated with yeasts will frequently have a slightly fruity odor.

C. Bacteria

Bacteria are prokaryotic cells that are approximately one μm in diameter, with short to elongated rods with spherical or ovoid forms. Individual bacteria closely combine in various forms, according to genera. Some spherical shaped bacteria occur in clusters similar to a bunch of grapes (e.g., staphylococci). Other bacteria (rod-shaped or sphere-shaped) are linked together to form chains (e.g., streptococci). Certain genera of sphere-shaped bacteria are formed together in pairs, such as pneumococci. Sarcinia microorganisms form as a group of four. Other genera appear as an individual bacterium; some bacteria possess flagella and are motile.

Bacterial discolorations range from variations in yellow to dark shades, such as brown or black. Certain bacteria have pigmentation of intermediate colors—red, pink, orange, blue, green, or purple. Some bacteria cause unstable color pigments and may cause slime formation.

Some species of bacteria produce spores that are resistant to heat, chemicals, and other environmental conditions. Many of these sporeforming bacteria are thermophilic and produce a toxin that will cause foodborne illness.

D. Viruses

Viruses are infective microorganisms that are approximately 1/100 to 1/10 the size of bacteria and can be seen only with an electron microscope. They cannot reproduce outside of another organism and are obligate parasites of all living organisms, such as bacteria, fungi, algae, protozoa, higher plants, and invertebrate and vertebrate animals. When a protein cell becomes attached to the surface of the appropriate host cell, either the host cell engulfs the virus particle or the nucleic acid is injected from the virus particle into the host cell, as with bacteriophages active against bacteria.

Transmission of viruses may occur when food workers are carriers. An infected employee can excrete the organism through feces and respiratory secretions. Modes of transmission include coughing, sneezing, touching a runny nose, and not washing the hands after using the toilet. Iodophor sanitizers destroy viruses.

VI. MICROBIAL PROLIFERATION

Multiplication of microbial cells normally occurs by binary fission in a growth pattern according to the typical growth curve illustrated in Fig. 1. These phases are described next.

A. Lag Phase

After contamination, a period of adjustment (or adaptation) to the environment occurs, with a slight decrease in microbial load due to stress. This reaction, which is called the lag phase, is followed by limited growth in the number of microorganisms. The lag phase can be extended with less microbial proliferation if rigid temperature control is practiced. A longer lag phase increases the "generation interval" of microorganisms. Microbial proliferation may be retarded also by decreasing the number of microbes that contaminate food, equipment, or buildings. Rigid sanitation and hygienic practices can lower the initial count of microbes and extend the lag phase.

B. Logarithmic Growth Phase

This phase involves multiplication by binary fission, characterized by the duplication of components within each cell, followed by prompt separation to form daughter cells. The number of microorganisms increases to the point that when cells divide, the increase in number of microbes occurs at an exponential rate until some environmental factor becomes limiting. The length of this phase may vary from two to several hours. Logarithmic growth rate is affected by initial microbial load and environmental factors such as nutrient availability and temperature. Effective sanitation to reduce the microbial load can limit the number of microbes that contribute to microbial proliferation during this growth phase.

C. Stationary Growth Phase

During this phase, environmental factors such as nutrient availability, temperature, and competition from another microbial population can limit and retard growth rate. Growth rate is affected because the number of microorganisms is frequently large enough that their metabolic by-products and competition for space and nourishment decrease proliferation.

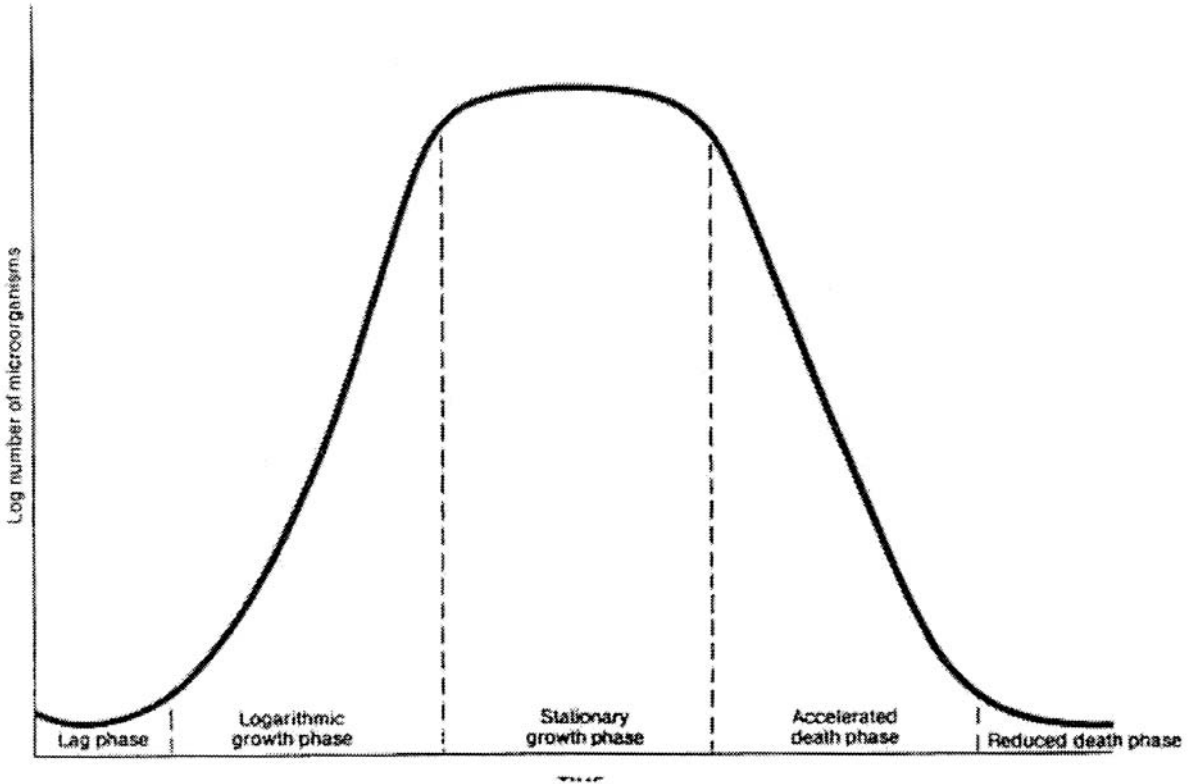

Figure 1 Typical growth curve of bacteria.

The length of this phase ranges from 24 hr to more than 30 days but depends on the availability of energy sources.

D. Accelerated Death Phase

Rapid death of microbial cells at an exponential rate in this phase occurs because of the lack of nutrients, effect of metabolic waste products, and competition from other microbial populations. The length of the accelerated death phase may range from 24 hr to 30 days depending upon temperature, nutrient supplies, microbial genus and species, age of the microorganisms, application of sanitation techniques and sanitizers, and competition from other microbial flora.

E. Reduced Death Phase

This portion of the microbial growth curve is caused by a sustained accelerated death phase so that the microbial population number is decreased to the extent that the death rate decelerates. After this phase, the organism has been degraded, sterilization has occurred, or another microbial population becomes established.

VII. ROLE OF BIOFILMS

Biofilms are microcolonies of bacteria closely associated with an inert surface attached by a matrix of complex polysaccharide-like material in which other debris, including nutrients and microorganisms, may be trapped. When a microorganism lands on a surface, it attaches itself with the aid of filaments or tendrils. The organism produces a polysaccharide-like material, which cements in a matter of hours the bacteria's position on the surface and acts as a glue to which nutrient material will adhere with other bacteria and, sometimes, viruses. The bacteria become entrenched on the surface, clinging with the aid of appendages.

A biofilm can add several layers of a polysaccharide material populated with microorganisms such as *Pseudomonas* and *Listeria*. Increased time of organism contact with the surface contributes to the size of the microcolonies formed, amount of attachment, and difficulty of removal. A biofilm will eventually become a tough plastic that often can be removed only by scraping. Although cleaned surfaces may be a sanitized, a firmly established biofilm has layers of organisms that may be protected from the sanitizer.

Biofilms are very difficult to remove during the cleaning operation. Microorganisms that appear to be more difficult to remove because of biofilm protection are *Pseudomonas* and *Listeria monocytogenes*. Current technology suggests that the application of heat appears to be more effective than that of chemical sanitizers, and Teflon appears to be easier to clear of biofilms than stainless steel. Caustics, bleaches, iodophors, phenols, and quaternary ammonium sanitizers do not effectively penetrate biofilms.

VIII. CONTAMINATION SOURCES

During animal harvesting and the processing, distribution, and food-service cycle, meat and meat products may be handled as many as 20 times. Because almost anything contacting meat and poultry can serve as a contamination source, the risk of this condition occurring rises each time that these products are handled. When alive, a healthy animal possesses defense mechanisms to counteract the introduction and growth of bacteria in the muscle

tissue. After harvest, the natural defenses break down, with a race between humans and microorganisms to determine the ultimate consumer. If handling is not hygienic, the microbes win.

Contamination sources are ubiquitous. Approximately one billion microbes are contained in a gram of soil attached to the hide of a live animal. A gram of manure contains approximately 220 million microorganisms. Initial contamination begins during harvesting. Exsanguination introduces bacterial contamination through the wound via the vascular system. Unwashed animals have approximately 155 million microbes per square centimeter of skin where the jugular vein is severed.

Even though the temperature of a scalding vat is approximately 60°C, the microbial load is approximately one million bacteria per liter of water. The dehairing operation for swine is responsible for microorganisms being beaten into the surface skin.

The stomach and intestinal contents are loaded with microorganisms; ruminant fluid averages 1.3 billion microorganisms per milliliter.

Carcass surface counts of microorganisms average 300 to 3000 per square centimeter. Beef and pork trimmings contain 10,000–500,000 bacteria per gram, depending on contamination and hygienic practices. Cutting boards on fabricating tables normally contain approximately 77,500 bacteria per square centimeter. Slicers, conveyors, and packaging equipment may increase the contamination of processed meats by 1000–50,000 bacteria per gram, depending on hygienic practices.

IX. PATHOGEN CONTROL

Meat and poultry products account for approximately 23% of foodborne illness outbreaks. Meat and poultry contribute to approximately 10% and 5%, respectively, of the reported deaths from foodborne illness.

The design of poultry processing equipment, especially the plucking equipment, is such that adequate cleaning is very difficult. The major risk in evisceration is the spilling of the gut contents onto the carcasses. Furthermore, the knives and hands of meat inspectors are often heavily contaminated. Regardless of the method of harvesting, the contamination rate of heavily infected poultry flocks may be 100% for the finished product. Immersion chilling poses a contamination threat because of entrapment of microorganisms in skin channels with the swelling of collagenous material in the neck flap area. The rinsing of poultry carcasses removes a small amount of microorganisms such as *Salmonella* and *Campylobacter* that may be present. In addition to hygienic practices that will be discussed later, pathogen reduction may be enhanced through cleaning of roofs over meat manufacturing areas. Processing equipment and exhaust stacks may be vented through the roof. If feasible, roof-mounted processing equipment should be enclosed with a floor to separate it from the processing area. Particles, especially hygroscopic powders, can deposit on the roof, especially if it is flat. This area may attract birds, rodents, or insects, which are carriers of pathogenic microorganisms. Pools of water will encourage these pests. A minimum slope of 1% is recommended for roofs to ensure drainage.

Since listeria growth can occur in some cooked meat products after packaging and a significant portion of fresh meats used as raw materials for processed products, the importance of the prevention of post-processing contamination of thermal processed products is evident. *Listeria monocytogenes* is frequently found around wet areas and floors, drains, ceiling condensate, mops and sponges, brine chillers, and at peeler stations. The control of *Listeria* organisms in meat processing plants is essential to reduce the potential of postprocessing contamination. The growth of this pathogen cannot be controlled

through refrigeration at 4°C (a common storage temperature) because this microbe can survive at 0°C storage environment. Use of antimicrobial agents, reduced temperature storage (less than 2°C), reformulation of products with reduced water activity, or post-processing pasteurization may be needed for the control of such psychotropic pathogens in meat products.

Pathogens can be controlled more effectively through the reduction of cross-contamination. Employees who work in the raw and finished product areas, such as smokehouses and water- and steam-cooking areas, should change outer clothing and sanitize their hands or change gloves when moving from a raw to finished product area. Utensils and thermometers that are used for raw and finished products should be sanitized each time they are used. Frequent cleaning with floor scrubbers is essential. If ceiling condensate is present, it should be removed by vacuum equipment. Cleaned floors that do not dry before production start-up should be vacuumed or squeegeed.

X. HYGIENIC DESIGN

The following suggestions should be considered for the hygienic design of meat and poultry plants:

1. Plant layout should discourage the entry of pests and vermin and control the movement of *Listeria monocytogenes* between raw and cooked product areas.
2. Air movement and refrigeration equipment should be designed for easy cleaning and sanitizing. Ready-to-eat areas should have a positive air pressure design.
3. All equipment and other surfaces should contain smooth, nonporous surfaces that are easily cleaned and sanitized.
4. Floors should be surfaced with materials that are easily cleaned and will not encourage water accumulation.

XI. SANITATION PRACTICES

All meat plant personnel should practice appropriate personal hygiene. Employees should wear freshly laundered clothes and avoid meat and processing equipment if they are ill. All cleaning and sanitizing compounds should be kept in an area accessible only to the sanitation supervisor, manager, and superintendents, and allocated only by the sanitation supervisor. Misuse of these compounds will inhibit effective cleaning and possibly result in personal injury and equipment damage. The water temperature should be locked in at 55°C. Cleaning compounds should be applied according to instructions or recommendations provided by the vendor.

The sanitation supervisor should inspect all areas nightly while the cleanup crew is on duty. All soiled areas should be recleaned prior to morning inspection.

The following sanitation practices, including the control of *Listeria monocytogenes*, are suggested to enhance a hygienic operation:

1. A sufficient number of employees, time, and supervision should be provided for effective cleaning and sanitizing.
2. Written cleaning and sanitizing procedures should be developed and posted in the plant for each area.
3. Environmental sampling programs to verify the effectiveness of cleaning and sanitizing should be established.

4. Mechanically or manually scrub floors and drains daily.
5. Drains should have a "quat" plug or be rinsed with disinfectants daily.
6. Scrub walls weekly.
7. Clean the exterior of all equipment, light fixtures, walls and ledges, piping, and vents.
8. Clean cooling and heating units and ducts weekly.
9. Caulk all cracks in the ceilings, walls, and other areas where they may occur.
10. Keep passageways that are common to raw and finished products clean and dry.
11. Minimize traffic in and out of processing and packaging areas and establish plant traffic patterns to reduce cross-contamination from feet, containers, pallet jacks, pallets, and fork trucks.
12. Change outer clothing and sanitize hands or change gloves when moving from a "raw" to a finished product area.
13. Require employees to change into clean washed clothes each day. Provide a color pattern to designate the various plant areas.
14. Require visitors to change into clean clothes provided at the plant.
15. Enclose processing and packaging rooms so that filtered air comes in and maintain these areas under positive pressure.

XII. SANITATION PRINCIPLES

Hygienic design and an effective cleaning operation can reduce labor costs up to 50%. Floors, walls, and ceilings should be constructed of impervious material that can be easily cleaned. Floors should be sloped with a minimum of 10.5 mm/m. Because soil from meat and poultry is primarily fat and protein deposits, a hot water wash is not an effective cleaning method. Hot water can loosen and melt fat deposits but tends to polymerize fats, denature proteins, and complicate removal of protein deposits by binding them more tightly to the surface to be cleaned. Other limitations include increased labor and water condensation on equipment, walls, and ceilings. It is difficult to remove heavy soil deposits through a hot-water wash, but other cleaning equipment is available, as will be discussed.

A. High-Pressure, Low-Volume Cleaning

High-pressure, low-volume spray cleaning effectively cleans difficult-to-reach areas with less labor. Portable units that can be easily moved throughout the plant and can be utilized for cleaning parts of equipment and building surfaces are especially effective for conveyors and processing equipment when soaking operations are impractical and hand brushing is difficult and labor-intensive. This cleaning technique also exists as a centralized high-pressure, low-volume cleaning system and is more expensive than the portable units, but requires less labor and maintenance. A major convenience of the centralized system is that quick and easy connections are located strategically throughout the plant. Figures 2 and 3 illustrate portable and centralized high-pressure, low-volume cleaning equipment.

B. Foam Cleaning

Foam is beneficial for cleaning large surface areas and can be used to clean transportation equipment exteriors, ceilings, walls, piping, belts, and storage containers. Portable foam equipment is similar in size and cost to portable high-pressure units. This cleaning method is popular in the meat and poultry industry primarily because less labor is involved.

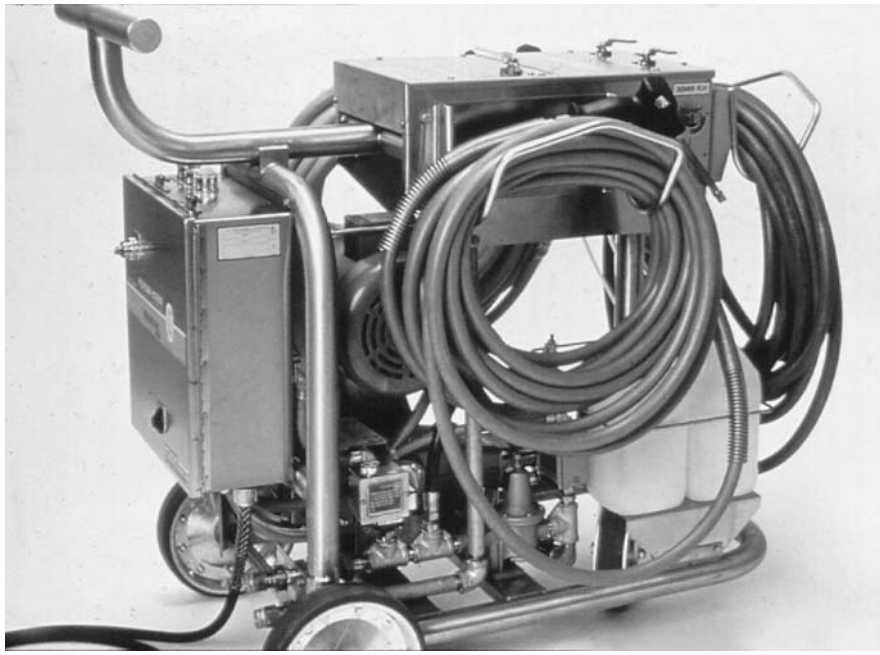

Figure 2 A portable high-pressure, low-volume cleaning unit that can be used where a centralized system does not exist. This unit is equipped with racks for hoses, foamer, and cleaning compound storage and provides two rinse stations and a sanitizer unit. Two workers can simultaneously prerinse, clean, postrinse, and sanitize. This equipment can also apply foam if the spray wand is replaced with a foam wand accessory. (Courtesy of Ecolab, Inc., Mendota Heights, Minnesota.)

C. Gel Cleaning

This equipment is similar to foam units, except that the cleaning compounds are applied as a gel. Gel is especially effective for cleaning packaging equipment because it clings to the surfaces and enhances soil removal. Equipment cost is similar to that of portable high-pressure or foam units.

D. Combination Centralized High-Pressure, Low-Volume, and Foam Cleaning

This equipment is similar to centralized high-pressure installations except that foam can also be applied to the equipment. This approach offers the most flexibility because foam can be used on large-surface areas, and high pressure can be applied to belts, conveyors, and difficult-to-reach areas in a meat or poultry plant.

E. Cleaning-in-Place (CIP)

This system incorporates a recirculating cleaning solution that is applied by installed nozzles, which automatically clean, rinse, and sanitize equipment. The use of CIP equipment in the meat and poultry industry is limited because it is expensive and lacks effectiveness in heavily soiled areas. This cleaning technique has some application in vacuum thawing

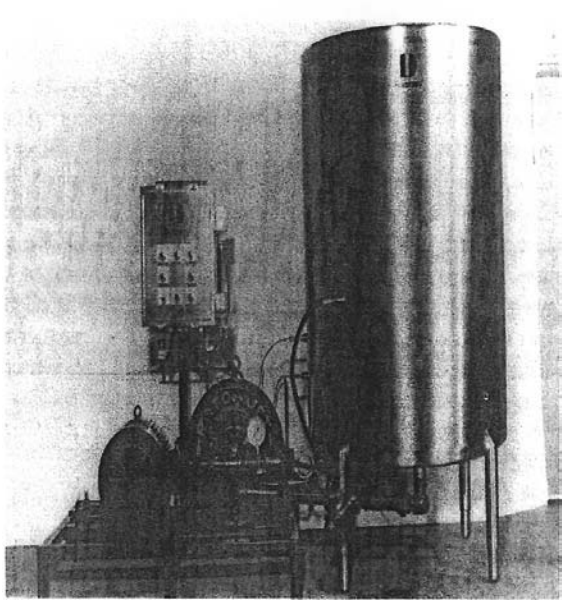

Figure 3 A centralized high-presure, low-volume system for a large cleaning operation. (Courtesy of Diversey Lever, Cincinnati, Ohio.)

chambers, pumping and brine circulation lines, preblend/batch silos, and edible and inedible fat-rendering systems.

XIII. CLEANING COMPOUNDS FOR MEAT AND POULTRY PLANTS

A. Strongly Alkaline Cleaners

These cleaners are incorporated only in areas with very heavy and difficult-to-remove soils such as smokehouses and certain rendering equipment. Examples of strongly alkaline cleaners are sodium hydroxide (caustic soda) and some silicates. The addition of silicates reduces corrosiveness and improves the penetration and rinsing properties of sodium hydroxide.

B. Heavy-Duty Alkaline Cleaners

These are the most frequently used cleaners for meat and poultry plants. They are incorporated in CIP, high-pressure, and other mechanized system as found in meat and poultry plants. The active ingredients of these cleaners may include sodium metasilicate, sodium hexametaphosphate, trisodium phosphate, and sodium pyrophosphate. The addition of sulfites tends to reduce the corrosion attack on tin and tinned metals.

C. Mildly Alkaline Cleaners

Mildly alkaline cleaners are frequently in solution and are used for hand cleaning lightly soiled areas (including hands) in meat and poultry plants.

D. Strongly Acid Cleaners

These compounds are corrosive to concrete, most metals, and fabrics. Strongly acid cleaners are used in cleaning operations to remove encrusted surface matter and mineral scale frequently found on steam-producing equipment, boilers, and some processing equipment. Strongly acid compounds used for cleaning operations in meat and poultry plants include hydrochloric (muriatic), hydrofluoric, sulfamic, sulfuric, and phosphoric acids. Nitric and sulfuric acids are not used in manual cleaners because of their corrosive properties. Corrosion inhibitors such as potassium chromate for nitric acid solutions or butylamine for HCI detergents may be added. Phosphoric acid and hydrofluoric acid both clean and brighten certain metals. However, hydrofluoric acid is corrosive to stainless steel and dangerous to handle because of the tendency toward hydrogen evolution during use. Phosphoric acid is widely used in the United States. It is relatively low in corrosive properties, compatible with many surfactants, and is used in manual and heavy-duty formulations.

E. Mildly Acid Cleaners

These compounds are mildly corrosive and may cause allergic reactions. Examples of mildly acid cleaning compounds are hydroxyacetic, acetic, and gluconic acids. The organic acids are higher in cost than are the other acid cleaning compounds. These mild compounds can also function as water softeners.

F. Neutral Cleaners

These cleaners, which have a pH near 7.0, are not applied much in meat and poultry plants. They are made from earth, including volcanic ash, pumice, silica flours, and feldspar and may be found in cleaning powders or pastes used in manual scrubbing and scouring operations.

G. Enzyme Cleaners

These compounds are increasing in popularity and offer potential for soil removal. Because they have not been used as much as other cleaners, their track record is less definitive.

XIV. SANITIZERS FOR MEAT AND POULTRY PLANTS

To maximize benefits from the use of a sanitizer, it must be applied to surfaces that are free of visible soil because some sanitizers react with residual organic matter. The soils that are more likely to nullify the effects of some sanitizers include fats, meat juices, blood grease, oil, and mineral buildup. These deposits provide areas for microbial growth, both below and within the soil, and can hold food and water necessary for microbial proliferation. Chemical sanitizers cannot successfully penetrate soil deposits to destroy microorganisms. Potential sanitizers will be discussed as classified.

A. Steam

This is an effective sanitizer for most applications; but is expensive because of the energy required to heat water and create steam. Unfortunately, operators can mistake water vapor

for steam and fail to provide adequate exposure to create a sanitizing effect. Steam should not be used in refrigerated areas because of condensation and energy waste, and it is unsatisfactory for continuous sanitizing of conveyors.

B. Chemical Sanitizers

Chlorine is one of the halogens used for sanitizing equipment, utensils, etc. The inorganic chlorine compounds most frequently used are described next.

1. Sodium and Calcium Hypochlorite

These sanitizers are more easily applied than elemental chlorine. Hypochlorous acid is an effective germicidal agent, and the activity of hypochlorites is pH dependent. Hypochlorites are economical and fast-acting but have limited residual properties and can cause metal corrosion and irritation to the hands.

2. Liquid Chlorine

This sanitizer is for use in the chlorination of processing and cooling waters to prevent bacterial slimes.

3. Chlorine Dioxide

This bactericide can be effective in the presence of organic matter because it does not react with nitrogenous compounds. It provides a longer residual effect than chlorine but is most effectively generated on site.

4. Active Iodine Solutions

Iodophors are very stable products with much longer shelf lives than hypochlorites and are active at a low concentration. They penetrate effectively and their acidic nature prevents film formation and spotting on the equipment. Solution temperature should be below 48°C because free iodine will dissipate.

5. Quaternary Ammonium Compounds (Quats)

These sanitizers are widely applied on floors, walls, equipment, and furnishings of meat and poultry plants. Quats are effective on porous surfaces because of their penetration ability. A bacteriostatic film that inhibits bacterial growth is formed when quats are applied to surfaces. Those sanitizers and compounds containing both an acid and a quat sanitizer are most effective in controlling *Listeria monocytogenes* and mold growth. Quats may be used temporarily when a mold buildup is detected. They are noncorrosive and nonirritating to the skin, and have no taste or odor in solutions. The quats are low in toxicity and can be neutralized or made ineffective by using any anionic detergent.

The quats have limited destructiveness, including ineffectiveness against most gram-negative microorganisms except *Salmonella* and *E. coli*. Furthermore, they are film forming on meat-handling and meat-processing equipment.

6. Acid Sanitizers

Acid sanitizers are considered toxicologically safe and biologically active and are frequently used to combine the rinsing and sanitizing steps. Organic acids such as acetic, peroxyacetic,

lactic, propionic, and formic acid are most frequently used in meat sanitation. The acid neutralizes excess alkalinity that remains from the cleaning compounds, prevents formation of alkaline deposits, and sanitizes. These compounds are especially effective on stainless steel surfaces or where contact time may be extended and have a high antimicrobial activity against psychrotropic microorganisms.

These sanitizers are fast-acting and effective against yeasts and viruses. A pH level of below 3 is most ideal for the performance of acid sanitizers. These sanitizers have good wetting properties and are nonstaining and usually noncorrosive, permitting exposure to equipment overnigh. Hard water and residual organic matter do not have a major effect on the ability of acid anionic sanitizers to destroy microorganisms, and they can be applied by CIP methods or by spray, or they can be foamed on if a foam additive is incorporated. Because acid sanitizers can lose their effectiveness in the presence of alkaline residuals or in the presence of cationic surfactants, all cleaning compounds should be rinsed from surfaces before acid sanitizers are applied.

The development of automated cleaning systems in food plants, where it is desirable to combine sanitizing with a final rinse, has made the use of acid sanitizers more attractive. Although these compounds are sensitive to pH change, they are less prone to be affected by hard water than the iodines. Nonfoaming acid synthetic detergent sanitizers are available to enhance drainage of the sanitizers from the equipment, thus increasing the utility of acid sanitizers for the food industry. Acids are not as efficient as irradiation and, when applied at high concentrations, can cause slight discoloration and odor on meat surfaces. Experiments with acetic acid have revealed a lack of effectiveness in the reduction of *Salmonella* contamination and thermoduric microorganisms.

Carboxylic acid sanitizers are effective over a broad range of bactericidal activity. Carboxylic acid is known as a fatty acid sanitizer and is composed of free fatty acids, sulfonated fatty acids, and other organic acids. They are stable in dilutions, in the presence of organic matter, and at high temperatures. These sanitizers are noncorrosive to stainless steel, provide a good shelf life, are cost-effective, and act as a sanitizer and acid rinse. Carboxylic acid is less effective against yeasts and molds and not as effective above pH 3.4–4.0 as some chemical sanitizers. This sanitizer is corrosive to nonstainless steels, plastics, and some rubbers.

7. Acid-Quat Sanitizers

This sanitizer is effective against *Listeria monocytogenes*. A limitation of this sanitizer is that it is expensive when compared to the halogens.

8. Hydrogen Peroxide

This antibacterial agent may be used on all types of surfaces, equipment, floors and drains, walls, steel mesh gloves, belts, and other areas where contamination exists, and it is effective against biofilms. Furthermore, this sanitizer has been demonstrated to be effective against *Listeria monocytogenes* when applied to latex gloves (2). A hydrogen peroxide solution may be used by itself or in combination with other processes to treat food-contact surfaces.

9. Ozone

This molecule acts as an oxidant and disinfectant and may be used as a control tool for microbial and chemical hazards. Common by-products of ozonation are acids, aldehydes, and ketones.

10. Glutaraldehyde

This sanitizer can control the growth of gram-negative and gram-positive bacteria, as well as species of yeasts and filamentous fungi found in conveyor lubricants used in the meat industry. When added to certain lubricant formulations, glutaraldehyde can reduce bacterial levels by 99.99% and fungal levels by 99.9% within 30 min.

XV. RECOMMENDED SANITARY WORK HABITS

Sanitation workers should follow the following general practices provided by Marriott (1):

1. Store personal equipment in a sanitary location and keep storage lockers clean.
2. Wash and sanitize utensils when they contain excessive soil and store them in a sanitary container that will not be in contact with flowers, clothing, lockers, or pockets.
3. Avoid product contact with surfaces not sanitized for meat and poultry handling. If any particle contacts the floor or other unclean surfaces, it should be washed thoroughly.
4. Use only disposable towels to wipe hands or utensils.
5. Cover hair to prevent product contamination from falling hair.
6. Remove aprons, frocks, gloves, or other clothing items that may come in contact with the product before entering employee welfare facilities.
7. Avoid production areas when a communicable disease, infected wound, cold, sore throat, or skin disease exists.
8. Do not use tobacco in any production area.

XVI. SANITATION PROCEDURES

Documentation of cleaning procedures is essential and especially important when supervision changes are made and for the training of new employees. Detailed cleaning operations should be written and posted in the plant. Every cleaning application should be adapted to the specific operation. Hoses and other equipment should be cleaned and returned to their proper locations after clean-up. Examples of cleaning procedures appropriate for specific operations can be found in *Principles of Food Sanitation, 4th edition* by Marriott (1).

Examples of selected areas will follow.

A. Receiving and Shipping Area

Frequency: Daily.

Procedure:

1. Cover all electrical connections, scales, and exposed product with plastic sheeting to prevent water and chemical damage.
2. Rinse the walls and floors with 50–55°C high-pressure water. The wall-rinse motion must be from top to bottom and side-to-side, with extraneous matter worked to the floor. A prerinse removes heavy soil deposits and wets the surfaces.
3. Apply an acid cleaning detergent through a slurry or foam gun. Recommended spray temperature is 55°C or lower. High-pressure output (for this and other

cleaning operations) is 25–70 kg per square centimeter and 7.5–12 liters per minute at the wand.
4. Within 20 min of the cleaning compound application, apply high-pressure rinse with 50–55°C water.
5. Remove, clean, and replace drain covers in the proper position after rinse-down.

B. Processed Products Storage Cooler

Frequency: Weekly. Products should be rotated for weekly cleaning of a section.

Procedure:

1. Clean each section, when empty, with a reliable floor cleaner. Apply a slurry or a foam via high pressure.
2. Rinse thoroughly with 55°C or lower temperature water at high pressure within 20 min of cleaning compound application. Work all debris to the floor from overhead fixtures and walls.
3. Squeegee the floor where water has accumulated to prevent it from freezing.
4. Remove, clean, and replace drain covers.

C. Processed Products Production Area

Frequency: Daily.

Procedure:

1. Dismantle all equipment and place the parts on a table or rack.
2. Pick up all large debris and deposit it in a receptacle.
3. Cover all electrical connections with plastic.
4. Prerinse all soil surfaces with 55°C water. Start at the top of all equipment and hose down to the floor. Avoid direct hosing of electrical equipment.
5. Following wash-down and subsequent heavy soil removal, apply an alkaline cleaner through a centralized or portable foam or high-pressure, low-volume system, using 55°C water. All tables, equipment undersides, and other difficult-to-access areas should be reached. Soak time prior to rinse-down should be 10–20 min. Foam penetrates less effectively but is a viable cleaning medium and is easily applied.
6. Rinse all equipment within 20 min after cleaning compound application. Using the same prerinse pattern as with the prerinse and detergent application, spray 50–55°C water on each side of each piece of processing equipment.
7. Thoroughly inspect all equipment surfaces and touch up as necessary.
8. Apply a sanitizer to all clean equipment with a centralized or portable sanitizing unit.
9. Remove, clean, and replace drain covers.
10. Apply white edible oil only to surfaces subject to rust or corrosion.

D. Processed Products Packaging Area

Frequency: Daily.

Procedure:

1. Dismantle all equipment and place the parts on a table or rack.

2. Remove large debris from the equipment and floor and place it in a receptacle.
3. Cover packaging equipment, motors, outlets, scales, and controls with plastic film.
4. Briefly prerinse all surfaces with 55°C water to remove heavy debris and to soak exposed surfaces. Hoses should be guided to force all debris toward the closest floor drain.
5. Apply an alkaline cleaner through centralized or portable high-pressure, low-volume cleaning equipment using 55°C water. Foam, gel, or slurry may be incorporated to introduce the cleaning compound. Cleaning compound application must cover the entire area—equipment, floors, walls, and doors.
6. Rinse the area and equipment within 15 to 20 min after cleaning compound application, using the same pattern of movement as when applying the cleaner.
7. Inspect the area and all equipment. Touch up as needed.
8. Remove, clean, and replace drain covers.
9. Sanitize clean equipment with an organic sanitizer and a centralized or portable unit.
10. Apply white edible oil only to surfaces subject to rust or corrosion.

E. Brine Curing and Packaging Area

Frequency: Daily.

Procedure:

1. Pick up all large debris and place it in a receptacle.
2. Cover all electrical connections, scales, and exposed product with plastic.
3. Prerinse the area and all equipment with 55°C water.
4. Place and acid cleaner in the shrink tunnel (if used), and circulate for approximately 30 min during prerinsing.
5. Rinse the shrink tunnel (if present) before cleaning compound application.
6. Place all prerinsed debris in a receptacle.
7. Apply an alkaline cleaner through a foam or slurry cleaning system, using 55°C water.
8. Rinse with 55°C water within 20 min after cleaning compound application.
9. Inspect the area and equipment and touch up as needed.
10. Remove, clean, and replace drain covers.
11. Sanitize all clean equipment with an organic sanitizer using a centralized or portable sanitizing unit.
12. Apply white edible oil only to surfaces subject to rust or corrosion.

F. Dry Curing Areas (Curing, Equalization, and Aging)

Frequency: Clean after product input and at the end of the designated cure or equalization period. To reduce mold growth in aging rooms, use filtered air or air conditioning with a filter.

Procedure:

1. Sweep floors.
2. Remove pallets and other portable storage equipment, and rinse cure and debris with 50°C water.

3. Hose down vacated areas with 50°C water.
4. Clean trolleys, trees, and other metal equipment used as outlined for wire pallets and metal containers.
5. Sanitize cleaned areas according to manufacturer requirements with a quaternary ammonium compound for its residual effect.
6. Spray aging rooms once every 3 months with a synergized pyrethrin to reduce insect infestation. Follow application directions on the pesticide label.

G. Smokehouses

Frequency: After the end of each smoke period.

Procedure:

1. Pick up large debris and place it in a receptacle.
2. Apply an alkaline cleaning compound recommended for cleaning smokehouses through a centralized or portable foam system. A high-pressure unit may be used where foam cannot penetrate (Fig. 4).
3. Rinse the area within 20 min after cleaning compound application. Start at the ceiling and walls, and work all extraneous matter to the floor drain.
4. Inspect all areas, and touch up where needed.
5. Apply an iodophor or quaternary ammonium sanitizer, with a centralized or portable sanitizing unit, around the entry area to reduce air contamination.

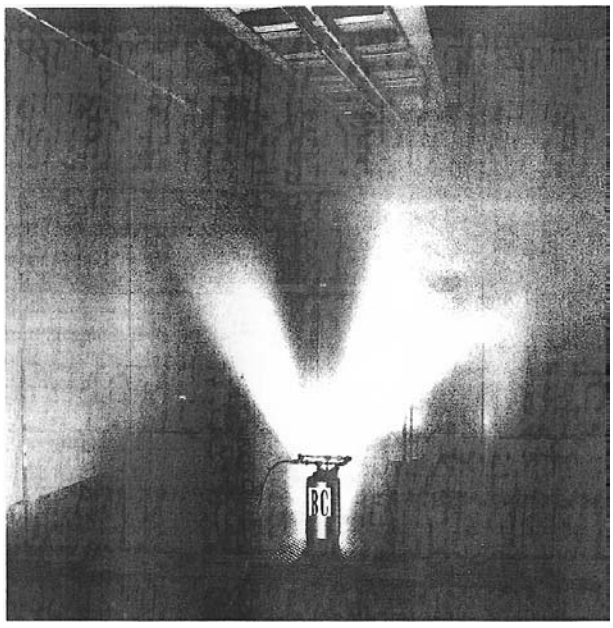

Figure 4 A portable atomizer that covers all stainless steel surfaces in a smokehouse and reduces cleaning time by 60–75%. (Courtesy of Birko Chemical Corporation.)

H. Smokehouse Blower

Frequency: After each use cycle.

Procedure:

Blades

1. Remove the blower housing access panel and drain plugs, soaking them with an alkaline solution.
2. Start the blower and flush with steam.
3. Stop the blower and flush again with water.
4. Repeat the operation until the equipment is clean.

Housing

1. Soak the inside of the plenum well, and wash the blower evolute wall with an alkaline cleaning solution.
2. Flush the housing with steam, then with water until it is clean.
3. Replace the drain plugs and access panel.

I. Smokehouse Steam Coils

Frequency: Depends on amount of use.

Procedure:

Coils

1. Open the chamber access door and soak with an alkaline cleaning solution, brushing vigorously wherever possible.
2. Flush the coils with steam, then with water. Repeat until the metal is shining.

Chamber around coils

1. Brush the cleaning solution or the inside of the chamber walls.
2. Use 55°C water to flush the chamber wall clean.
3. Close the coil chamber access door.

J. Smokehouse Ducts and Nozzles

Frequency: Depends on amount of use.

Procedure:

Outside ducts

1. Remove the ductwork at the back of the house as well as carbon deposits. Disassembly is not necessary if the ducts have access panels.
2. Spray the inside surface with an alkaline cleaning solution.
3. Flush the outside ducts clean with 90°C water or steam, followed by hot water. Repeat this exercise until the metal is exposed.

Inside return ducts

1. Mark the positions of the slide panels over the return ports so that the panels may be set back to their original openings for a balanced house.

2. Open the ports all the way and use as access doors for applying an alkaline cleaning solution to the ducts.
3. Use 90°C water for flushing the return ducts. Repeat this operation until the metal is exposed.
4. Reset the slide panels to the originally marked positions.

Inside jet ducts

1. Open side access panels (or drop hinged panel, depending on type of house).
2. Soak the inside ducts and nozzles with a cleaning solution.
3. Use 90°C water to flush these ducts clean. Repeat this operation until the metal is exposed.
4. Close the access panel (or hinged panel).

Exhaust stack

1. Disassemble the stack (or open access panels).
2. Soak the stack interior with an alkaline cleaning solution.
3. Flush the stack with 90°C water or steam, followed by hot water. Repeat this process until the metal is exposed, especially in the damper area.
4. Reassemble the stack (or close the access panels).

K. Smoke Generator

Frequency: Depends on amount of use.

Procedure:

Filter

1. Soak the filter in an alkaline cleaning solution.
2. If mineralization has occurred, cut the frame apart, and clean the leaves individually. Reweld the frame after cleaning. Avoid warping.

Baffle and cascade chamber

1. Mechanically or brush manually the baffles (especially the edges) with a wire brush.
2. Scrape the edges of the cascade water outlet.

Wash chamber

1. Disassemble the duct connecting the smoke generator to the house.
2. Remove soot and ash from the chamber below the filter.
3. Clean the duct and chamber surface until the metal is exposed.

L. Spiral Freezer

Frequency: After use.

Procedure: See instructions for specific equipment to be cleaned.

1. To minimize friction, regularly wash the spiral with a foaming cleanser.

2. When the track is warm, wipe with a cloth dampened with a cleaning compound solution. If the track is cold, a dry cloth may be used. Tie the cloth to the underside of the conveyor belt and let it be drawn through the spiral.
3. Defrosting the evaporator coil alone is insufficient for cleaning. Coils may appear clean, but grease, oils, salts, food adjuncts, and organic materials often remain hidden on internal surfaces. Therefore, it is necessary to clean and sanitize contaminated sites with warm water and a pH-balanced cleaning compound. Cleansing solutions typically include an etching agent, a degreaser, inhibitors, metal protectors, stabilizers, and water. A mildly alkaline cleanser is recommended for cleaning the evaporator coil.
4. If the freezer has been supplied with a recirculating CIP system, use a low-foaming detergent. Otherwise, use a high-foaming detergent. A chemical supplier should be consulted to determine the best chemical cleanser.

M. Wash Areas

Frequency: Daily.

Procedure: See instructions for specific equipment to be cleaned.

1. Use a separate wash area for raw and cooked product equipment to reduce the spread of *Listeria* and spoilage microorganisms.
2. Locate this operation in an area where clean equipment does not cross fresh product areas of the plant.

N. Wire Pallets and Metal Containers

Frequency: Prior to use.

Procedure:

1. Use high-pressure 55°C or lower water as a prerinse.
2. Preferably, apply an alkaline cleaning compound with a foam unit. If foam is unavailable, use a high-pressure, low-volume unit. Whichever method is incorporated, never spray more containers than can be rinsed before the cleaning compound dries.
3. Use a high-pressure spray of 55°C water as a rinse.
4. Inspect all rinsed containers and clean again as needed.

O. Offices, Locker Rooms, and Restrooms

Frequency: Offices, daily; locker rooms and restrooms, at least every other day.

Procedure:

1. Cover electrical connections with plastic sheeting.
2. Clean areas with a foam or high-pressure unit (or scrub brush and/or mop).
3. Within 20 min after cleaning compound application, rinse with 55°C water.
4. If the cleanser and rinse cannot clean dirty areas or if drains are not present, hand scrub with scouring pads.

P. Garments

Frequency: Daily.

Procedure:

1. Place dirty garments into the washer-extractor. Do not load the washer beyond its rated capacity.
2. Place the programmer dial at the start of the cycle and push the "On" and "Run" selector buttons. The drum programmer will automatically select the wash time and water temperature. Cleansing compounds are dispensed into the wash cycle immediately. Many detergents are available for this application. An example would be the mixture of 1 kg of a commercial laundry compound and 0.25 kg of chlorine bleach per 65 kg of dry weight load. Bleach should not be used when washing gloves.
3. After the wash-extractor cycle, remove the garments and place them in the dryer. Set aside garments not thoroughly clean for rewashing. Do not load the dryer beyond its rated capacity.
4. Set the temperature at 121°C for 30 mins. Dry gloves for only 20 min.
5. Place dried garments in a clean wire crib or equivalent container. They do not need to be folded.

SUMMARY

Effective sanitation in meat and poultry plants involves the control of microorganisms that cause food spoilage and foodborne illness. An efficient cleaning operation can reduce labor costs in meat and poultry plants by up to 50%. The optimal cleaning system depends on soil type and equipment to be cleaned. Although high-pressure, low-volume cleaning equipment is known to be the most effective for removing heavy organic soils, foam cleaning has become more prominent because it is easier and quicker. CIP systems are typically limited primarily to applications that involve large storage containers because of higher investment costs and more cleaning limitations. Acid cleaning compounds are used more frequently to remove mineral deposits. Alkaline cleaning compounds are more effective in the removal of organic soils than other cleaners. Chlorine compounds are the most effective and economical sanitizer for the destruction of residual microorganisms. However, iodine compounds cause less corrosion and irritation, and quaternary ammonium sanitizers

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Fermented Soy Foods: An Overview

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I. INTRODUCTION

The soybean belongs to the family Leguminosae. The cultivated form, named *Glycine max* (L.) Merrill, grows annually. Soybean seeds are spherical to long oval. Most of them are yellow, but some are green, dark brown, purplish black, or black.

Historical and geographical evidence indicates that the soybean originated in northern China. Its cultivation in the region started as early as the new stone ages—some 5000 years ago. Soybean (then known as *shu*, now as *da dou* or *huang dou* in Chinese) was repeatedly mentioned in later records and was considered one of the five sacred grains, along with rice, wheat, barley, and millet (1).

During the course of soybean cultivation, the Chinese had gradually transformed soybeans into various forms of soy foods. Tofu, soy paste (jiang), soy sauce, and soy sprouts are among the popular ones. This transformation makes soybeans as a food more versatile, more tasteful, and more digestible. Because of their high content of protein and oil, with a fairly balanced amino acid profile and abundant essential fatty acids, the traditional soy foods have nourished Chinese peoples from their creation to the present.

Along with soybean cultivation, methods of soy food preparation were gradually introduced to Japan, Korea, and some other Asian countries about 1100 years ago. Peoples in these regions not only accepted soy foods, but also modified them and even created their own types to suit the local taste. Japanese natto and Indonesian tempeh are just two examples.

Soybeans were first introduced to Europe and then North America in the 18th century. However, large-scale official introduction into the United States did not occur until the early 1900s. Thousands of new varieties were brought in, mostly from China, during this period. Until 1954, China led the world in soybean production. Since then, the United States has become the world leader.

In the past century, the soybean emerged as one of the most important agricultural commodities in the world, with a steady increase in annual production. Currently, global production is estimated at 150 million metric tons, with major producers being the United States, Brazil, Argentina, China, and India. One of the major reasons that the soybean is an important world agricultural commodity is its unique chemical composition. On average, dry soybean contains roughly 40% protein, 20% oil, 35% carbohydrate, and 5% ash. Thus, soybean has the highest protein content among cereal and other legume species and the second highest oil content among all food legumes. Other valuable components found in

soybeans include phospholipids, vitamins, minerals, and isoflavones. No wonder soybeans are known as "yellow jewel" or "miracle beans."

The art of preparing soy foods has now spread to the rest of the world, thanks to agricultural and processing innovation, cultural exchanges, and the influence of Chinese and other Asian immigrants. More important, for the past decade, medical research has revealed many health-promoting effects of soy foods, including cholesterol reduction, cancer prevention, improved vascular health, preserved bone mineral density, and alleviation of menopausal symptoms (2–6). As a result, consumers' interest in soy foods has reached an all-time high. Many companies and institutions are looking into making and promoting soy foods. Many modern processing technologies are being used in making soy foods. These have led to large-scale production and distribution of soy foods in many regions of the world, with improved and consistent quality.

This chapter provides a general overview of fermented soy foods in term of product types, microorganisms involved, preparation principles, and utilization. The four chapters that follow will cover individual products in detail. Additional information on the subject can be found in Wang et al. (1), Shi and Ren (7), Liu (8), and Han et al. (9).

II. TYPES OF FERMENTED SOY FOODS

There are four major fermented soy foods (soy paste, soy sauce, tempeh, and natto) and three minor fermented soy foods (sufu, soy nuggets, and soy yogurts). Table 1 lists English names, local names, and general description and uses of fermented soy foods.

Fermented soy foods vary greatly in terms of microorganisms involved, methods of preparation, length of fermentation, principles, and end uses. It takes only a few days to prepare tempeh and natto, but preparation of the remaining types of fermented soy foods generally requires several months to complete. Except for natto and soy yogurts, which result from bacteria fermentation, all others are fermented mainly through fungal fermentation. In terms of end uses, most fermented soy foods, including soy paste, soy sauce, soy nuggets, and sufu, are generally served as seasonings in cooking or making soups. They contribute more flavor than nutrition to the diet. They are characterized by high salt content added during the second stage of fermentation, as well as the presence of certain by-products (such as acids, alcohols) from desirable fermentation. Both salt and by-products inhibit or slow down spoilage of these products and allow them to have a relatively long shelf life. The remaining types, including tempeh, natto, and soy yogurts, contain no added salt and are consumed as a part of a main meal. Thus, they contribute protein and oil to the diet besides their characteristic flavor. Approximate chemical composition of selected Asian soy foods is listed in Table 2.

There are also some similarities among some of these fermented soy foods. For example, fermented soy paste, soy sauce, and soy nuggets share the same type of microorganisms, *Aspergillus* sp. In addition, most fermented soy products start with whole soybeans that have been soaked and cooked. Only soy yogurt and sufu derive from soymilk and tofu, respectively.

III. SOY PASTE (JIANG AND MISO)

Soy paste is an important fermented soy food in Asia. It has a color varying from a light, bright yellow to very blackish brown, a distinctively pleasant aroma, and a salty taste. Soy

Table 1 Names, General Description, and Utilization of Fermented Soy Foods

Soy foods	Native names			General description	Uses
	Chinese[a]	Japanese	Others[b]		
Soy sauce	Jiang you (Chiang yu)	Shoyu	Kecap (In., Ma.) Tayo (Ph.) Kang jiang (Ko.)	Whole soybeans (or defatted soy flakes) and wheat fermented with *Aspergillus*, *Pediococcus*, *Torulopsis*, and *Zygosaccharomyces*. Dark brown liquid, salty and meaty.	All-purpose seasoning for dishes or soups
Soy paste	Dou jiang, Jiang (Chiang)	Miso	Tauco (In.,Ma.) Tao si (Ph.)	Whole soybeans with wheat flour, rice, or barley, fermented with *Aspergillus*, *Pediococcus*, *Zygosaccharomyces*, *Torulopsis* & *Streptococcus*. Light yellow to dark paste, salty & meaty.	All-purpose seasoning for dishes or soups
Tempeh	Tian bei	Tempe	Tempeh Kedelai (In.) Tempeh (Ma.)	Cooked and denulled soybeans fermented with *Rhizopus*. Soft beans bound by white mycelia, cakelike, nutty flavor.	Fried or cooked as part of meal, snack, or in soups
Natto	Na dou, Shui dou chi	Natto		Cooked whole soybeans fermented with *Bacillus* natto. Soft beans covered by viscous, sticky polymer, distinct aroma.	Seasoned and eaten with cooked rice
Soy nuggets	Dou chi (Toushih)	Hamanatto	Tao si (Ph.)	Whole soybeans fermented with *Aspergillus*. Soft beans with black color, salty and meaty taste	Cooked with vegetable and meat or served as seasoning
Sufu, Chinese cheese	Dou furu, Furu (Tou fu ju))			Tofu femented with *Actinomucor* or *Mucor*. Creamy, cheeselike, salty, distinct aroma.	A condiment, served with or without further cooking
Sour soymilk	Suan dou nai			Lactic fermentation of soymilk	Frozen dessert
Soy yogurt					Yogurt replacement

[a] Mandarin Chinese (or Cantonese).
[b] In. = Indonesian, Ko. = Korean, Ma. = Malaysian, and Ph. = Filipino.

Table 2 Proximate Composition of Some Traditional Soy Foods (Fresh Weight Basis)[a]

Soy food	Moisture	Protein	Fat	Soluble carbohydrate	Fiber	Ash
Soybeans (10)	12.0	34.3	17.5	26.7	4.5	5.0
Soymilk (7)	88.8	3.2	3.6	3.9	0.0	0.5
Soft tofu (7)	90.3	5.3	0.9	2.5	0.1	0.9
Firm tofu (7)	84.0	10.7	2.1	2.0	0.3	0.9
Deep-fried tofu (7)	45.2	24.6	20.8	7.5	0.4	1.5
Okara (7)	87.0	2.6	0.3	7.6	1.8	0.7
Yuba (7)	7.1	50.5	23.7	15.3	0.3	3.1
Rice miso (light) (10)	50.0	12.6	3.4	19.4	1.8	12.8
Rice miso (red) (10)	50.0	14.0	5.0	14.3	1.9	14.8
Soybean miso (10)	47.5	16.8	6.9	13.6	2.3	13.0
Chiang (Chunky) (11)	48.6	11.6	5.2	27.2	2.1	7.4
Tempeh (12)	64.0	18.3	4.0	11.0	1.7	1.0
Natto (10)	58.5	16.5	10.0	10.1	2.3	2.6
Sufu (red) (7)	55.5	14.6	5.7	5.8	0.6	17.8
Sufu (white) (7)	56.5	14.4	11.2	4.8	0.7	12.4

[a] Expressed as g/100 g.
Source: Refs. 7, 10 through 12.

paste is commonly known as *jiang* (Mandarin) or *chiang* (Cantonese) in China, *miso* in Japan, *jang* in Korea, *taucho* in Indonesia, and *taotsi* in the Philippines.

Developed in China some 2500 years ago, jiang was the progenitor of the many varieties of soy paste and soy sauce that are now used throughout the world (11). It was believed to have been introduced by Buddhists to Japan in the sixth century, along with the introduction of Buddhism. During the first several centuries after introduction, Japanese jiang, which was known as *hishio* at the time, was very similar to Chinese jiang, and many of the basic raw materials and complex fermentation techniques were undoubtedly acquired from China. Later, the Japanese gradually altered the basic ingredients and preparatory procedures and transformed Chinese jiang into Japanese miso. As a result of this gradual transformation, Chinese jiang and Japanese miso are now quite different from each other in terms of raw materials, appearances, taste, and utilization. Miso is made from soybeans mixed with rice or barley, or from soybeans alone, whereas Chinese soy jiang is often made from soybeans and wheat. Miso is a paste resembling peanut butter in consistency and may have a sweet taste. However, Chinese jiang may be unground so that individual particles of soybeans are present.

In terms of end uses, jiang in China is used as a base for sauces served with meat, seafood, poultry, or vegetable dishes. In Japan, however, miso is mainly dissolved in water as a base for various types of soups. Often, the soup contains vegetables, algae, tofu, meat, or fish. Other seasonings such as monosodium glutamate may also be added. Furthermore, jiang in China has a broader meaning than miso in Japan. In addition to fermented soy paste, it refers to other types of fermented paste made with such materials as wheat and seafoods.

The method for making miso and jiang may vary with variety, but the basic steps and principles are essentially the same. For example, preparation of rice miso consists of five steps: preparation of rice koji, treatments of soybeans, mixing of all ingredients, fermentation, and pasteurization and packaging.

The word *koji* is a Japanese word meaning bloom of mold. Its Chinese counterpart is called *qu*. Made by growing molds on rice, barley, wheat, soybeans, or a combination, koji contains a great variety of enzymes that digest starch, protein, and lipid components in raw materials. It is used for making not only jiang and miso but also some other fermented products, such as soy sauce, soy nuggets, and Japanese sake.

The microorganisms found in koji almost always belong to the fungi species *Aspergillus oryzae* and/or *A. sojae*. The genus *Aspergillus* belongs to Fungi Imperfecti. These fungi reproduce only asexually by means of specialized spores called conidia, which are borne at the tips of special fungal filaments known as conidiophores. The koji mold has the ability to utilize starch, oligosaccharides, simple sugars, organic acids, and alcohols, as carbon sources, and protein, amino acids, and urea, as nitrogen sources.

A. oryzae is aerobic, with growth optima generally at a pH of 6.0, a temperature of 37°C, and a water content of 50% in a medium. When air supply is limited, or water content of the medium is below 30%, its growth slows down. When the temperature is below 28°C, its growth also becomes slow but enzymatic activities remain high. At the optimum growth temperature, however, its enzymes become less active (7).

For making Japanese rice miso, rice koji is made through fermenting cooked rice with a koji starter containing *Aspergillus oryzae* spores at about 30°C for a few days. Treatments of raw materials include soaking and heating. Soaking hydrates proteins and other grain components and thus facilities subsequent heating. Insufficient hydration leads to insufficient denaturation of soy protein as well as softening of soybean texture during the subsequent heat treatment. The effects of heating include denaturing proteins so that they can be utilized either by koji mold or hydrolyzed by koji enzymes, inactivating trypsin inhibitors and hemagglutini, softening the texture of soybeans, sterilizing soybeans, and removing the unpleasant bean odor (13,14). Wheat flour is often steamed or roasted before use.

During koji making, spores of the koji mold germinate and grow into mycelia and eventually sporulate. As a result, various enzymes are produced. Major enzymes include protease, amylase, glutaminase, lipase, hemicellulase, pectinase, and esterase. Among them, the activities of the first three enzymes are most important in determining the quality of the final product. Starch from rice, barley, or wheat is converted by amylase into dextrin, maltose, and glucose, which contribute sweet taste to the miso. Protein from soybeans is converted by proteases and peptidases into water-soluble nitrogen compounds consisting mainly of oligopeptides and amino acids. The amino acids, particularly glutamic acid, contribute the delicious taste to miso (15,16). At the same time, oil from soybeans is hydrolyzed in part by lipase to free fatty acids and glycerol.

In addition, some enzymes produced by koji molds are yet to be discovered. For example, Kudou et al. (17) reported that *A. oryzae* used in the fermentation of miso and soy sauce also produces an enzyme known as soybean saponin hydrolase that is capable of hydrolyzing soybean saponins. Other enzymes may have multiple functions. For example, Tomita et al. (18) found that glutaminase purified from koji mold, *A. oryzae* MA-27-IM, could also catalyze gamma-glutamyltranspeptidation reaction, leading to formation of gamma-glutamyl peptides in the final product.

As the enzymatic digestion progresses, it generates fermentable substances such as simple sugars for growth of yeasts and lactic bacteria that came from inoculum. Under anaerobic conditions, these organisms further break down sugars to acid, alcohols, and other substances. In a survey of commercial samples of both miso and soy sauce produced in Japan, Ibe et al. (19) reported that most samples contained nonvolatile amines, including tyramine, histamine, and phenethlamine. It was believed that these amines were produced by

microorganisms during fermentation because they were not detected from such raw materials as koji and soybeans. Later, Ibe and coworkers (20) isolated tyramine- and histamine-producing bacterial strains from miso and identified the tyramine strain as *Enterococcus faecium* and the histamine strain as *Lactobacillus* sp.

As fermentation and aging continue, there are complex chemical and biological interactions among various components in miso (21). In general, acids react with alcohols to produce esters, which contribute a distinct aroma to miso. Amino acids and sugars interact to produce browning substances, which contribute in part to the color of miso. Amino acids play the dual role of enhancing flavor and darkening color, so miso with the richest color is often considered to be richest in flavor.

Comparing preparation methods of jiang and miso, however, reveals two major differences. The first difference lies in composition of raw material used for making koji; in making rice or barley miso, koji is made from rice or barley alone; in making soybean miso, koji is made from soybean alone; in making jiang, however, koji is made from a mixture of soybeans and wheat flour. The second difference reflects the degree of koji maturation. Koji for jiang is usually incubated for a longer time and harvested when the koji mold has sporulated. In contrast, koji for miso is harvested before the mold starts to sporulate. Therefore, it contains white mycelium only.

IV. SOY SAUCE

Soy sauce is a dark-brown liquid made from a mixture of soybeans and wheat, mostly through natural fermentation. It is known as *jiangyou* (Mandarin) or *chiangyu* (Cantonese) in China, meaning oil from *jiang* (a fermented food paste), and *shoyu* in Japan.

Discovered in China more than 2500 years ago, soy sauce is one of the world's oldest condiments. As an all-purpose seasoning, soy sauce offers a wide range of applications. It not only contributes a unique flavor profile to traditional Asian foods but also holds great potential as a flavoring and flavor-enhancing material for a wide variety of non Asian food products (22). Today, it is becoming increasingly known in the West as natural seasoning that promotes balance among ingredients in food products.

New studies show that many soy sauce components also contribute an antioxidant effect when applied to food (23,24). They also contribute an umami taste. Umami is the fifth flavor, coined by the Japanese, added to four basic flavors—sweet, salty, sour, and bitter. Often translated as "savory" or "brothy," umami can be described as the tongue-coating, meaty flavor of sautéed mushrooms, a juicy steak, or a rich stock (25). Furthermore, soy sauce is found to show antiplatelet activity and its alkaloidal components are responsible. Therefore, it can be considered a functional seasoning (26).

There are many types of soy sauce. Based on preparation principles, soy sauce is divided into three groups, fermented soy sauce, chemical soy sauce, and semi chemical soy sauce. Based on geographical location, there are Chinese and Japanese soy sauces. Based on physical or other properties, there are liquid soy sauce, powdered soy sauce, clear soy sauce, reduced salt soy sauce, and preservative free soy sauce.

Just as in the making of other soy foods, preparation of soy sauce was once a family art passed down from one generation to the next. At present, production of soy sauce at a domestic level is still popular in some regions of the world, but the majority is made in commercial plants. There are great variations in methods of making soy sauce, depending on geographic regions and varieties of soy sauce. However, regardless of the level of production and methods used, the basic steps and principles involved are very similar. Generally

speaking, the process consists of treatment of raw materials, koji making, brine fermentation, pressing, and refining (8,27–29).

Soy sauce and soy paste not only have the same Chinese origin but also bear many similarities in their preparations and principles. These include the microorganisms involved, treatments of soybeans, preparation of koji, and fermentation. As in soy paste, the major microorganism involved in soy sauce is *Aspergillus* sp. Just like making jiang or miso, there are two fermentation occurring in soy sauce preparation. The first fermentation is solid state and occurs during koji making where various enzymes are produced under aerobic conditions. The second fermentation occurs after addition of brine, known as brine fermentation. It is mainly anaerobic. At the earlier stage of brine fermentation, enzymes from koji hydrolyze proteins to yield peptides and free amino acids. Starch is converted to simple sugars, which in turn serve as substrates for growth of various types of salt resistant bacteria and yeasts. These organisms become dominant in sequence as fermentation progresses. All these enzymatic and biological reactions, together with concurrent chemical reactions, lead to the formation of many new volatile and nonvolatile substances that contribute to the characteristic color, flavor, and taste of soy sauce (28,30,31).

However, there are some major differences between soy paste and soy sauce. First, soy sauce is a liquid extract pressed from fermented mixture of soybeans and wheat, and only the pressed and filtered liquid is consumed, whereas soy paste is a product of fermented soybeans mixed with wheat, rice, or barley, and the whole product is consumed. Consequently, two additional steps—pressing and clarification—are involved in soy sauce making. Second, during the second stage of fermentation, more water is used in soy sauce production and fermentation time is longer for soy sauce production. As a result, there is a complete digestion of soybean proteins, carbohydrates, and some other components. Many amino acids are produced, particularly glutamic acid, which is responsible for delicious taste. Nearly 300 kinds of volatile components have been identified as flavor contributors in soy sauce. Among them are 37 hydrocarbons, 30 alcohols, 41 esters, 15 aldehydes, 5 pyrones, 25 pyrazines, 7 pyridines, 11 sulfur compounds, 3 thiazoles, 3 terpenes, and 8 other miscellaneous compounds. The most important components of soy sauce flavor seem to reside in its weak acidic fraction, including 4 hydroxyfuranones; many phenolic compounds, such as 4-ethylguaiacol, 4-ethylphenol, and 2-phenylethanol; and some alcohols and esters, such as maltol, furfuryl alcohol, and ethyl acetate (28,32).

V. SOY NUGGETS

Soy nuggets are known as "*douchi*" in mandarin Chinese, or "*toushih*" in Cantonese. They are made by fermenting whole soybeans with strains of *Aspergillus oryzae*, although some other strains of fungi or bacteria may also be responsible. The product usually has a flavor similar to soy sauce or jiang. Because the color of the beans is often black after fermentation, the product is also known as salted black beans in the West.

Soy nuggets are commonly used as an appetizer to be consumed with bland food, or as a flavoring agent to be cooked with vegetables, meats, and seafoods. Its production is very limited. There is limited literature available regarding the soy nugget (7,8).

Originating in China before the Han dynasty (206 B.C.), douchi, or soy nuggets, is the first soy food to be described in written records. The Historical Records (or *Shi zi*) written by Si Ma Qian, who died about 85 B.C., mention ".... a thousand jars of leaven or douchi." Therefore, soy nuggets might be the oldest fermented soy food ever developed by mankind (11).

In Japan, a similar product known as hamanatto is produced, especially in the vicinity of Hamanatsu, Shizuoka Prefecture, from which the name of the product was perhaps derived. Similar products are also produced in some other countries of Asia. For example, in the Philippines, one is known as *tao-si*; in eastern India, as *tao-tjo*.

To make soy nuggest, whole soybeans are soaked 5–6 hr, and then steamed or boiled in water until soft, normally for about 2 hr. The cooked beans are inoculated either naturally or with a koji starter. When koji is matured, it is washed with water. Washing helps removal of extra mycelium, spores as well as contaminants, and ensures that the finished product is shining and free of mold odor and bitter taste. After washing, koji is mixed with a certain amount of water, salt, and spices before being put into a jar for fermentation. Fermentation normally is carried out under natural conditions for several months. Sometimes, soy sauce is used in place of brine. In other regions, spices, wine, or sugar may be added at this stage. The aged beans become wet soy nuggets, which may be further dried to make dry soy nuggets. The finished product consists of intact beans with a blackish color, and it has a salty taste and soy sauce flavor. Because of relatively high salt and low water contents, the product can be kept for a long time (7). The principles for making soy nuggets as well as the microorganisms involved are similar to those of fermented soy paste or soy sauce.

VI. TEMPEH

Tempeh, or tempe in some literature, is another fermented soy food. Widely believed to have originated in Indonesia centuries ago, tempeh is made by fermenting dehulled and briefly cooked soybeans with mold, *Rhizopus*. Freshly prepared tempeh is a cakelike product, covered and penetrated completely by white mycelium, and has a clean, yeasty odor. When sliced and deep-fried, it has a nutty flavor, pleasant aroma, and crunchy texture. Unlike most other fermented soy foods, which are usually used as flavor agents, tempeh serves as a main dish or meat substitute.

Also, unlike most other fermented soy foods, which usually involve long-time fermentation and complex processes, tempeh preparation is short and simple.

Although relatively unknown in the surrounding countries such as Thailand, China, and Japan, where soybeans form an important part of the diet, tempeh is one of the most popular fermented foods in Indonesia, New Guinea, and Surinam. Because of its meat-like texture and mushroomy flavor, tempeh is well suited to Western tastes. It is becoming a popular food for vegetarians in the United States and other parts of the world.

Traditionally, making tempeh is a household art in Indonesia (12). The method of preparation varies from one household to another, but the principal steps are basically the same. Soybeans are cleaned and then boiled in water for 30 min to loosen the hulls, which are then removed either by hand or by rubbing with feet, and washed away with water. The dehulled beans are soaked overnight to allow full hydration and lactic acid fermentation. The soaked, dehulled beans are cooked again for 60 min and drained using woven-bamboo baskets, then spread on a flat surface for cooling to room temperature. In certain places, soybeans are soaked in water until the hulls can be easily removed by hand and washed away, and then boiled until soft, normally for at least 30 min. This avoids twice-cooking procedures.

The cooled treated beans are now inoculated with a starter culture or an inoculum from a previous batch, wrapped in banana leaves or perforated plastic bags, approximately a quarter pound per package. Fermentation is allowed to occur at room temperature for up to 18 hr, or until the beans are bound by white mycelium. Alternatively, inoculated beans are

spread on shallow aluminum foil or metal trays with perforated bottoms and covered with layers of banana leaves, waxed paper, or plastic films that are also perforated.

Although *R. oligosporus* is the principal species used in Indonesia for tempeh fermentation, numerous bacteria of both spore- and nonsporeforming types exist in tempeh. Yeast and other microorganisms are also found. Saono et al. (33) isolated 69 mold species, 78 bacteria species, and 150 yeast species from 81 tempeh samples. They found that bacteria are undesirable because their unchecked growth during fermentation contributes off odor. Mulyowidarso et al. (34) also reported variable growth of bacteria (10^8–10^9 cfu/g) during fermentation of soybeans into tempeh. *R. oligosporus*. *Bacillus pumilus*, and *B. brevis* were the predominant bacterial species, but *Lactobacillus casei*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* also contributed to the fermentation. Steinkraus et al. (35) reported that certain bacteria acidify soybeans during soaking, which helps mold growth during subsequent fermentation. Thus, to enhance the role of the bacteria during soaking, traditional tempeh makers usually add water from previous soaking into the new batch.

There are many aspects of changes during tempeh fermentation, including temperature, pH, and chemical composition of the soybean substrate. All of these are brought about by microbial growth and enzyme actions. As microorganisms grow, they produce various enzymes that break down soybean components. This leads to some compositional changes,—although, compared with miso and soy sauce, these changes are much less vigorous due to limited production of enzymes by the tempeh mold. In general, in comparison between tempeh and unfermented dehulled soybeans, there are some increases in levels of free amino acids and free fatty acids, a slight decrease in oil content, and no significant changes in protein and ash contents. In addition, there are significant increases in contents of several vitamins, including riboflavin, vitamin B_6, nicotinic acid, pantothenic acid, biotin, and folacin. Some of these increases are of several-folds, although thiamin was found to change little (36). Furthermore, in some tempeh, there is formation of vitamin B_{12}, which is widely attributed to the presence of certain strains of contaminating bacteria, such as *Klebsiella pnemoniae* (37,38) and *Citrobacter freundii* (38).

VII. NATTO

Originating in the northern part of Japan about 1000 years ago, natto is one of the few products in which bacteria predominate during fermentation. When properly prepared, natto has a slimy appearance, a sweet taste, and a characteristic aroma. In Japan, it is often eaten with soy sauce or mustard, and served for breakfast and dinner along with rice. Similar products are found also in Indonesian and Thailand but not in China.

To make natto, soybeans, preferably small-seeded, are washed and soaked in water overnight (Fig. 1). The soaked beans are then cooked in a steamer or a pressure cooker for about 30 min or until the beans are soft. Cooked beans are now drained and cooled to about 40°C. Before the responsible bacterium was isolated, the cooked soybeans were traditionally wrapped with rice straw and set in a warm place for 1–2 days. Rice straw was credited not only for supplying the fermenting microorganism but also for absorbing the unpleasant odor of ammonia released from natto and imparting the aroma of straw to the product.

Ever since the isolation of the responsible microorganism, *Bacillus natto*, the old straw method has been largely abandoned in favor of pure culture fermentation. Instead of wrapping with rice straw, the cooked beans are now inoculated with a pure-culture suspension of *B. natto* and thoroughly mixed before being packed in wooden boxes or polyethylene bags. The polyethylene bags are perforated from the outset for good aeration.

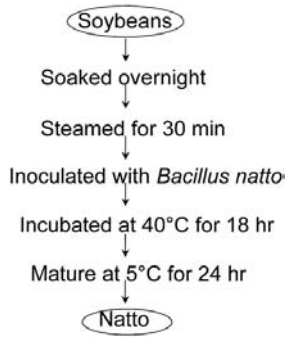

Figure 1 Natto production outline.

The packages are put into shallow sliced-wood or polystyrene trays and set in a warm thermostatic chamber with a controlled temperature at 40°C. After 14–20 hr of fermentation, the bacteria will have covered the beans with a white sticky coating, indicating the time for harvesting. Overfermentation should be avoided because it leads to release of ammonia, which not only spoils the natto flavor but also destroys the *B. natto* and promotes spoilage by other organisms.

For better quality, the package may be kept at a refrigerating temperature for 1–2 days to allow maturation and then taken out for consumption or retailing as needed. This practice is most common in the large plants.

Unlike preparations of many other fermented soyfoods, which are complex and require actions of multiple microorganisms with a mold dominating, preparation of natto is relatively simple and requires action of only one type of microorganism—bacteria, *Bacilus natto*. *B. natto* is known to be an aerobic, gram-positive rod and is classified as a related strain of *B. subtilis*. Many new strains of *B. subtilis* suitable for natto production have been bred and are available in markets (39–41).

During fermentation, *B. natto* bacteria grow, multiply, and sporulate. One of the most remarkable features of the genus *Bacillus* is the secretion of various extracellular enzymes, including protease, amylase, gamma-glutamyltranspeptidase (GTP), levansucrase, and phytase. *Bacillus natto* is known to produce large amounts of these extracellular enzymes. For example, proteases are known to be secreted 15 to 20 times more from *B. natto* than from other strains of *B. subtilis* (39), whereas the activity of gamma-GTP in *B. natto* was found to be 80 times higher than in regular strains of *B. subtilis* (42).

As natto bacilli grow, the enzymes they secreted or produced catalyze many chemical and enzymatic reactions that lead to production of the characteristic sticky materials as well as formation of the characteristic aroma and flavor. The viscous material consists of polysaccharide (a levan-form fructan) and gamma-polyglutamic acid. The formation of polyglutamate is believed to be catalyzed by gamma-GTP (42).

During natto fermentation, there are no significant changes in fat and fiber contents of soybeans during fermentation (44). There is also no significant change in fatty acid composition of soy lipids, indicating no lipase is produced by *B. natto* (43). However, soluble carbohydrate almost completely disappeared. Sucrose, raffinose, and stachyose decreased from 8 hr onward. At the same time, glucose, melibiose, manninotriose, and a small amount of fructose were liberated. Glucose and fructose were then consumed completely before 14 hr whereas melibiose, manninotriose, and remaining stachyose remained unchanged in beans. Citric acid, the major organic acid in steamed soybeans, was decomposed quickly

in parallel with glucose decomposition, indicating that these are used as the carbon source by *B. natto* (44).

At the same time, many volatile components, which contribute the characteristic aroma and flavor of natto, are produced by the natto bacteria, including acetoin, 2,3-butanediol, and acetic, propionic, *iso*-butyric, 2-methylbutyric, and 3-methylbutyric acids. Their contents vary remarkably among samples, and with stage of fermentation and subsequent storage (44).

Recently, there have been new developments in natto research. One relates to studies showing the health benefits of natto. For example, natto has been shown to contain significant amount of vitamin K_2 (menaquione-7). Vitamin K_2 is the cofactor that converts nonactivated osteocalcin into activated osteocalcin by carboxylation. Vitamin K_2 is derived from the microorganism *Bacillus subtilis* (natto)itself. Consequently, in rat studies as well as in vitro, natto has been shown to promote formation of osteocalcin, a bone protein, and participate in bone formation (45,46).

Another development in natto research is production of natto with little smell. The product is coined as niowanatto in Japan. The specific natto smell is composed of several kinds of branched short-chain fatty acids such as butyric acid and 2-methylbutyric acid, which are formed through the metabolism of amino acids such as leucine, isoleucine, and valine. Takemura et al. (47) isolated a *B. natto* mutant lacking an enzyme that converts isoleucine. Using this mutant *B. natto* strain, niowanatto can be produced.

VIII. SUFU

When fresh tofu is fermented with a strain of certain fungi such as *Mucor hiemalis* or *Actinomucor elegans*, it becomes a new product known as sufu or Chinese cheese. The product consists of tofu cubes covered with white or yellowish-white fungous mycelia, it was firm texture, salty taste, and characteristic flavor. Although relatively unknown in some adjunctive countries such as Japan and Korea, sufu was produced in China long before the Ching Dynasty and is consumed mainly as an appetizer or relish by all segments of the Chinese population, including those living overseas.

Preparation methods vary with types of sufu and regions, but all involve three basic steps: preparing tofu, molding (first fermentation), and bringing (second fermentation) (7,48,49). The type of fungus involved in sufu fermentation has a great influence on quality of the finished product. In order to produce a sufu with good quality, the fungus must produce white or yellowish-white mycelia to warrant an attractive appearance. The texture of mycelial mat should also be dense and thick so that a firm film will be formed over the surface of the fermented tofu cubes to prevent any distortion in their shape. It is also essential that the mold produce abundant lipases and proteases to digest the protein and lipid-rich substrate—tofu. Furthermore, the mold should develop neither a disagreeable odor nor an astringent taste. Using pure culture fermentation, Wai and his coworkers found that all four species, *A. elegans*, *M. hiemalis*, *M. silvaticus*, and *M. subtilissimus*, meet these requirements and can produce sufu with good quality. However, *A. elegans* is the best among them, and therefore has been adopted for commercial production. Later, a mutant, *A. taiwanensis*, was also used as a commercial starter to produce sufu (50). In addition, some species of *Mucor* are widely used for commercial production of sufu in China (7).

According to a recent review on sufu (9), several types of sufu can be made through uses of different processing methods and addition of different color and flavor agents. For example, choice of processing can result in mold-fermented sufu, naturally fermented sufu,

bacterial fermented sufu, or enzymatically ripened sufu, and choice of dressing mixture can give red, white, or gray sufu.

IX. SOUR SOYMILK AND SOY YOGURT

Certain lactic acid bacteria have the ability to grow on dairy milk to produce various types of fermented dairy products, including acidophilus milk (sour milk), cultured buttermilk, yogurt, cheese, and other cultured milk products. The microbial action not only increases the shelf life and nutritional value of these products but also makes them more pleasant to eat or drink.

Soymilk resembles dairy milk in composition. Lactic acid fermentation of soy milk produces such products as sour soymilk, soy yogurt, or sogurt. Fermentation of soymilk offers not only a means of preserving soymilk but also a possibility for modifying or improving its flavor and texture so that it becomes more acceptable to Westerners. It also leads to new types of soy products that resemble cultured dairy products.

Studies show that certain lactic acid bacteria, such as *L. acidophilus*, *S. thermophilus*, *L. cellobiosis*, *L. plantarum*, and *L. lactis* have been shown to grow well in soymilk, but they produce less acid in soymilk than in cow's milk (51,52). The major reason is that soymilk lacks monosaccharides and the disaccharide lactose. Instead, it contains such sugars as sucrose, raffinose, and stachyose, which are not readily digestible by many lactic starters due to lack of alpha-galactosidase in these organisms. Therefore, efforts have been made to increase acid production during lactic fermentation of soymilk. These include selection of culture strain, alteration of processing conditions during soymilk preparation, addition of fermentable sugars; and/or enrichment with dairy ingredients (52,53). Recently, Ara et al. (54) identified a new lactic bacterium strain, *Streptococcus* sp. S85-4, and found it had an effect on improving some unpleasant taste, such as astringency, associated with fermented soymilk.

X. SUMMARY

Soybeans can be made into various types of soy foods, categorized either as nonfermented or fermented. Among the major fermented soy foods are soy paste, soy sauce, tempeh, natto, sufu, soy nuggets, and soy yogurts. Almost all of them are traditionally made in Asia. A few of them are now commercially available throughout the world with modern processing technology.

Fermented soy foods serve either as flavoring agents or as regular food. With our continuous efforts to understand the science behind the traditional fermentation practice, uncover the health benefits of soyfoods, search for exotic and ethnic flavors, and advance processing and packaging technology, it is anticipated that fermented Asian soy foods will gain further acceptance throughout the world.

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Soy Sauce: Manufacturing and Biochemical Changes

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I. INTRODUCTION

It is generally accepted that the Chinese invented soy sauce. Over the years, overseas Chinese introduced soy sauce to the different parts of the world. Later, the Kikkoman Company of Japan promoted soy sauce in various countries. For example, in the United States, the Kikkoman Company was the first major foreign company to build a large-scale processing plant to manufacture soy sauce. Now, its products are in almost every supermarket or grocery store in this country. Depending on the raw materials used and manufacturing method applied, there are many different kinds of soy sauce, and they differ in flavor.

Soy sauce is a liquid seasoning (condiment) of protein hydrolysate made by traditional fermentation, fast (modern) fermentation, or combined fermentation method (1). Soy sauce contains salt, sugars, alcohol, chemical seasonings, and/or chemical preservatives. Traditional fermented soy sauce utilizes (a) soybeans (*Glycine max*, Mill), defatted soybeans, or black beans and (b) wheat or rice as the raw materials. These raw materials are steamed or otherwise treated and inoculated with the cultured starter to form the soy koji. Rice may be steamed separately and/or saccharified and added to the soya koji. In addition, brine or raw soy sauce is added. Addition of cellulase can also be added to facilitate the fermentation. The mixture is then fermented and matured to form the clear seasoning (soy sauce).

In the "chemical method" of making soy sauce, the raw materials include (a) fermented soy sauce cake or raw soy sauce, (b) liquid acid/enzymatic hydrolysates of soy or other plant protein. They are fermented and matured accordingly to form the clear liquid seasoning (soy sauce). This product is also called modern fermented soy sauce or fast fermented soy sauce.

Composite soy sauce is a mixture of traditionally fermented soy sauce and the chemical soy sauce. It is also called amino liquid, enzyme-treated combined soy sauce, or amino soy sauce.

The common soy sauce is the product made from soybean, defatted soybean, and wheat or rice as the raw materials, utilizing one of the above-mentioned fermentation procedures.

Raw soy sauce is the liquid recovered by pressing the fermented soy paste without additional treatment.

Special soy sauce such as black bean soy sauce utilizes black beans, wheat, and rice as the raw materials. It is fermented by the traditional method.

In-yu utilizes black beans as the raw material to make the soy koji.

Light-colored soy sauce has color intensity (absorbance at 555 nm) less than 3, or standard color larger than 19.

Low-salt soy sauce has a salt content of less than 12% (based on chloride content) and contains no preservatives.

Soy sauce paste is soy sauce with thickener added to achieve a consistency of 250 cps at 25°C. These pastes include in-yu and "pot-bottom" sauce.

In Japan, koikuchi soy sauce is made with about equal amounts of soybeans and wheat as the raw materials.

Usukuchi soy sauce is made with soybeans or soybeans with small amount of wheat as the raw materials. In the mixing of ingredients step, addition of steamed rice or saccharified rice is optional in order to inhibit the color-darkening reaction of the soy sauce.

Tamari (supernatant juice) soy sauce is made from soybean only or soybean with small amount of wheat added as raw materials for soy koji.

Processed soy sauce is made from about equal amounts of soybeans and wheat, with raw soy sauce added to it. After fermentation, it forms a high-density soy sauce. It is also called sweet soy sauce.

Siro soy sauce is made from soybeans with only small amount of wheat as the raw material. At the same time, the color-darkening reaction is extensively inhibited.

Koikuchi soy sauce, usukushi soy sauce, tamari soy sauce, and black bean soy sauce are classified as fermented soy sauces. Chemical soy sauce and composite soy sauce are also called amino soy sauce.

II. PROCESSES FOR MANUFACTURING FERMENTED SOY SAUCE

Processes for the manufacturing of soy sauce in Taiwan are similar to those for Koikuchi soy sauce in Japan.

A. Raw Materials

Soy sauce is made from proteinaceous materials (soybean), starchy materials, and salt.

1. Proteinaceous Materials (Soybeans)

From the very beginning, soybean has been the proteinaceous material used for soy sauce fermentation (2). In the soy sauce manufacturing process, the soybean oil is not utilized. Therefore, the utilization of defatted soybean as the proteinaceous material instead of the whole soybean in soy sauce fermentation has been applied in the early development of the soy sauce industry. There are two kinds of defatted soybeans. One is the compressed defatted soybean, in which pressure is applied on steamed soybean to extract the soybean oil. The other is solvent-defatted soybean, in which the soybean oil is extracted from soybean by means of solvents. Solvent-defatted soybean is currently used as the raw materials for soy sauce manufacturing. Compressed defatted soybean can also be used to make good soy sauce. Composition of solvent defatted soybean is as follows (Table 1).

Solvent-defatted soybean contains a large quantity of protein. Among the amino acids, glutamic acid content is very abundant, about 20% of the total soy protein. Solvent-

Table 1 Chemical Composition of Solvent-Defatted Soybean (%)

Moisture	Crude protein	Carbohydrates	Crude fat	Crude fiber	Ash
11–13	47–51	19–22	0.1–1.5	3–5	4–6

Source: Ref. 1.

defatted soybean does not contain starch; the main components in the carbohydrate fraction are sugars and polysaccharides such as sucrose, raffinose, stachyose, neutral arabinogalactan, acidic polysaccharide, and arabinan. Solvent-defatted soybean also contains lecithin and other phosphates (2).

Because high temperature is applied in the solvent-defatting process, the proteins in the soybean have been extensively denatured. The water-soluble proteins are only 20–30% of the total as compared to 80% in the whole soybean before solvent extraction. However, the weak alkali-soluble proteins increase to 40–50%. This kind of protein is not easily decomposed by the microbial proteinase and poses a problem in the microbial digestion process. Fortunately, in the actual fermentation process, it does not differ much from the protein of the whole soybean.

2. Starchy Material

Wheat is the most suitable starchy material in soy sauce manufacturing (3). Other starchy materials such as rice, barley, maize, and milo can also be used. The chemical composition of wheat is as follows (Table 2).

Wheat contains only small amount of protein. However, the glutamic acid content is high among the amino acids in wheat protein, about 32% of the total protein. About 10% of the carbohydrates is pentosan, mainly xylan. This helps the formation of melanin on the coloration of soy sauce. Wheat also contains considerable amounts of phytin-type phosphates.

3. Salt

The crude salt commercially available has about 94% sodium chloride and is suitable for soy sauce manufacturing.

4. Starter Mold

The starter mold should possess the following properties: high unit spore number, high spore germination rate, low foreign mold count, suitability for dry preservation, abundant secretion of protease. The starter mold should be separately selected for suitable soy sauce or miso fermentation. The simultaneous use of *Aspergillus oryzae* with strong alkali protease and *Aspergillus sojae* with strong alfa-amylase as starter molds can increase the utilization rate of the raw material and result in a better-flavored product (4).

Table 2 Chemical Composition of Wheat (%)

Moisture	Crude protein	Carbohydrates	Crude fat	Crude fiber	Ash
10–14	8–15	68–74	1.8–2.0	2.5–3.5	1.4–2.0

Source: Ref. 2.

5. Water

The water used for soy sauce manufacturing should be regular tap water or water from wells and should not contain sanitizer(s) or large amount of iron compounds.

6. Additives

Suitable additives such as flavor enhancers, sweeteners, colorants, and preservatives are permissible. See sections below.

B. Manufacturing Process

Figure 1 is a generalized scheme for the manufacturing of soy sauce. The different steps are discussed in greater detail in the following section (5).

C. Treatments of the Raw Materials

1. Treatment of Defatted Soybeans

The main purpose of treating defatted soybeans is to denature the soybean proteins to a form so that it is more suitable for the protease from the starter to digest or hydrolyze (primary denaturation), without the presence of "native" proteins (6). However, the soy protein should also not be denatured to an extent that it is not easily utilized by the protease (secondary denaturation.) In order to avoid over-denaturation, the current practice is to steam the soybeans for less than one hour, followed by vacuum cooling to drop the temperature rapidly. The soybeans are also stirred slowly in the cooker to maintain even temperature in order to prevent soy proteins being denatured. This can be achieved by utilization of NK style pressurized rotating steam cooker and vacuum cooling equipment (Fig. 2) (7).

In order to achieve a moisture content of 60–62% in the cooked soybeans, cold water is added at a ratio of 120–130% on a weight basis of the soybeans (i.e., 120–130 kg of water per 100 kg of defatted soybeans). This amount of water is added by spraying the water through tubes inside the cooker with proper rotation of the cooker. After water spraying, the rotation is continued for 5–10 min to allow even water absorption. At the same time of the rotation of the cooker, pressurized steam is injected to 1.8 kg/cm^2 and held for 5 min (or 3 min at 2.0 kg/cm^2). The cooked soybeans are vacuum-cooled rapidly to 40°C. In a closed system mode, the cooled soybeans are moved to a sealed mixing tank equipped with a screw-type stirrer and mixed with broken and roasted (stir-fried) wheat, as well as the soy sauce starter (koji).

Another procedure is to inject steam during rotation of the cooker to achieve a steam pressure of 0.9–1.0 kg/cm^2 and maintain it for 40–60 min. After cooking, the pressure is reduced with a jet condenser to rapidly cool the cooked soybeans to ambient pressure in 10–25 min. When the temperature reaches 40°C, the broken and roasted (stir-fried) wheat and one-half of the starter (koji) are introduced to the cooker and mixed. This procedure can avoid the introduction of foreign microorganisms and contamination of the batch when cool air is introduced. If the water absorbed by the soybeans is insufficient or uneven, this is a cause of turbid soy sauce (8).

In the past, the cooking process usually took 7 hr at ambient pressure with a large amount of steam. If the soybeans are cooked for only 2–3 hr, are undercooked and the soy proteins are not denatured. It was fairly common to have turbid soy sauce products. Also, in

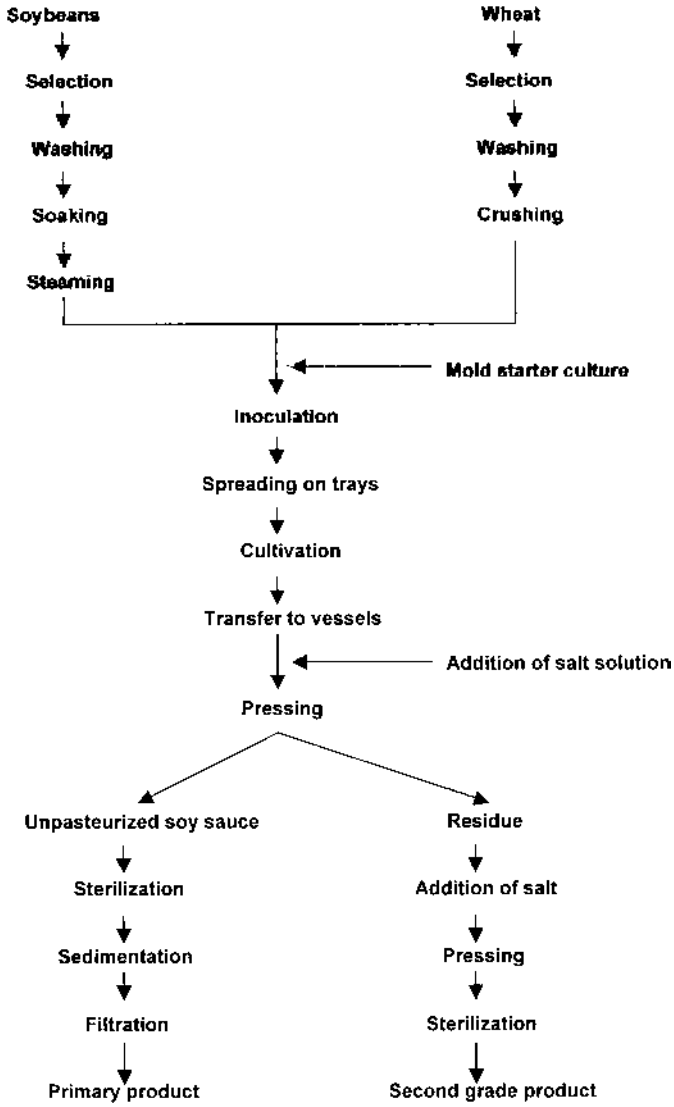

Figure 1 Flowchart of soy sauce manufacturing.

the past, the soybeans were pressure-cooked with steam at a pressure of 0.7–0.8 atm for 3–4 hr. No matter which way the soybeans were cooked, at ambient pressure or pressurized, the cooked soybeans were left in the cooker overnight. They were taken out the next morning. This procedure will soften the soybeans properly, and the soybeans are suitable for inoculation and making the mash (9).

The use of the NK-type rotating pressurized cooking process does not require the holding overnight period. It can be used for making the soy starter (koji) the same day. The nitrogen utilization rate is much higher than the traditional method, about 83% as compared to 65–70%.

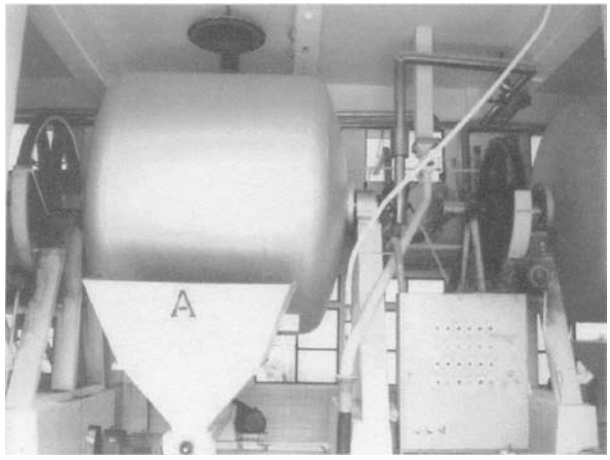

Figure 2 Soybean steamer.

2. Treatment of the Wheat

Roasting (stir-frying) the wheat has the following purposes: it (a) heat-gelatinizes the starch for easy hydrolysis and utilization by the microorganisms, (b) evaporates the water for easy breaking, and (c) kills the foreign microorganisms on the wheat surface.

After selection, the wheat is stir-fried in a flat pan, followed by breaking. For large-scale production, the roasting is conducted in an iron rotating drum (Fig. 3), followed by a roller breaker (Fig. 4). The breaking should achieve a status of 30% passing rate for a No. 30 mesh. This is to avoid contamination during preparation of soy starter (koji) and proper covering of the surface of moistened soybeans. The degree of roasting is to have fox (brown) color on the surface of the wheat kernels, with the disappearance of green odor. The volume of the kernels will increase considerably. Direct roasting is also conducted, avoiding burning or charring of the kernels. The bran should be slightly stir-fried or steam-cooked.

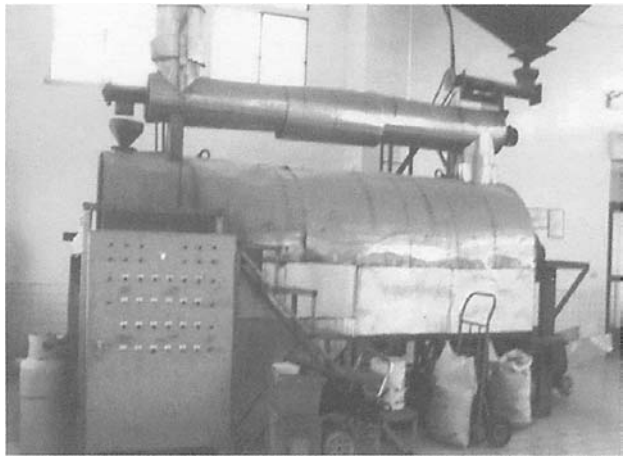

Figure 3 Wheat roaster.

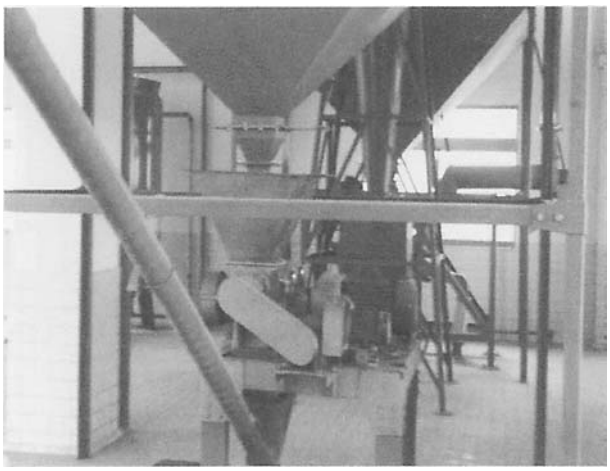

Figure 4 Wheat crusher.

3. Preparation of the Brine

The commercial salt is dissolved in cold water to the desired concentration. Brine concentration too high is undesirable for proper hydrolysis or fermentation. Concentration too low has the possibility of deterioration. A concentration of Baume 19–20° (22.5–23.7% sodium chloride, average 23.1%) is most suitable. The brine should be cooled to 0–5°C. When mixed with the soy (starter) koji, the mash liquid will have a temperature of about 15°C. This will adjust the pH of the mash liquid and increase the nitrogen solubility rate. At the same time, it can prevent deterioration caused by sedimentation and intensify the color.

D. Preparation of Soy Koji

1. Mixing of Ingredients

Cooked soybeans are spread and cooled on either concrete floors or wood panels. Broken and roasted wheat is spread on top of these cooled soybeans, followed by mixing. When the NK-style cooker is used, the cooked soybeans are mixed with the broken and roasted wheat inside the cooker, or introduced to a closed chamber for mixing. The ratio of defatted soybeans and wheat is 5:5 to 6:4. With this proportion, the broken and roasted wheat will absorb most of the moisture in the cooked soybean (about 60–62%), and allow the mixture to reach a moisture content of about 47%.

2. Traditional Starter (Koji) Making Procedures

a. Koji Room The beds in the koji room for making koji should be made of stainless steel with the exception of black bean soy sauce. The frames should be made of stainless steel or other corrosion-resistant materials. The double-walls of the koji room can be made of wood, bricks, concrete, lime panels, or thick panels, and can be filled with rice bran to maintain the temperature. The insulation materials can also be asbestos or aluminum foils. The floor in the room should be made of concrete. On one side of the room is a 1-meter-wide opening with easily opened double-sliding-doors. The room ceiling has 2–3 large

easily opened ventilators for the convenience of air exchange. The height of these windows (ventilators) should be 2.2 m. The ceiling temperature is maintained by electrical heating. Temperature of the incubation beds is maintained by passing steam through pipes on both sides of the beds. Lower racks are placed along the walls of the room for easy placements of koji trays.

b. Koji Trays The dimensions of the koji trays vary among locations. Wooden trays 60 cm long, 30 cm wide, and 7 cm deep are common. In recent years, large trays six times the size of the traditional trays or saran sheets are also used. They have the advantage of cleanliness and easy operation.

c. Mold Starter When the raw material for mold starter (cooked barley and wheat bran at ~1:1 ratio, or cooked brown rice and potash) is cooled to about 40°C, it is inoculated and mixed thoroughly. In order to ensure thorough mixing of raw material with small quantity of starter inoculum, a small quantity of broken and roasted wheat is mixed with the starter inoculum thoroughly before spreading onto the raw material. The inoculum is 0.01%. (The larger the inoculum, the shorter the starter preparation time, whereas the smaller the quantity, the longer the incubation time.) The inoculated mixture is then placed on starter trays or in the starter room to incubate, with spore number reaching 8×10^8/g. Two hours after inoculation, about half of the spores will germinate, with mycelia visible after 20 hr of incubation. The optimum incubation temperature is 37–38°C. The soya starter is different from rice starter: it can be used directly without sieving.

d. Preparation of Soya Koji After the mixing of the raw materials with the starter (ratio of starter to raw material is 0.1% for 4-day-old starter, and 0.2–0.3% for 3-day-old starter), the mixture is then piled on the koji trays. The piles should have the center being higher, about 20–30 cm. Pile height too high may cause temperature too high inside and is not easy to cool down. Therefore, the amount has to be suitable, with less in the summer and more in the winter. The piled koji mixture is then moved to the koji room previously adjusted to 28°C. Also, the room should have good air circulation, with even moisture and temperature control.

The temperature of the koji mixture will drop at the beginning. This is due to the moisture evaporation, drawing the latent heat out of the mixture. With the progression of mold multiplication, the temperature will increase again. After 20 hr of piling, the temperature reaches 38–40°C, and the mixture requires the first turnover. The purpose is to lower the temperature of the koji mixture, replenish the air, remove the carbon dioxide and other undesirable gases produced in the incubation, loosen up the caked mixture, provide more even distribution of the inoculum, and evaporate off the excess moisture. This process is usually conducted manually; at the same time several shallow horizontal trenches are created to provide more surface to lower the temperature. In order to provide even temperature for each tray, it is necessary to exchange the position of the trays, the upper and inside trays having higher temperatures and the outer and lower trays having lower temperatures. During the process, the ventilators at the ceiling are opened to adjust the moisture and temperature inside the room and to introduce fresh air. The temperature of the koji mixture is about 31–33°C.

During the initial koji starter–producing period, the temperature is maintained to encourage rapid mold growth. This fungal growth also at the same time produces heat that has to be removed to maintain the temperature not higher than 40°C. At temperatures higher than 45°C, growth of *Rhizopus*, *Mucor*, and *Bacillus subtilus* may occur and overproduce the koji starter. This will initiate the production of black koji, burnt koji (due to excessive temperature increase with inadequate turnover), natto koji (excess temperature increase causing the natto koji to produce natto odor and ammonia) and results in failure of

the soya koji production. Therefore, the bacteria number has to be controlled at 1000 organisms per gram or lower.

Five to six hours after the first turnover, the temperature will increase again to about 40°C. The mycelia on the surface will turn white with production of characteristic soya koji odor. This is the time for the second turnover. The purpose is the same as the first turnover, but with extra consideration of the air circulation and moisture evaporation. The koji mixture is again shallowly trenched. The temperature will increase again, but most of the moisture will be evaporated. Holding the koji mixture after the second turnover will not increase the temperature that much. When the temperature is over 43°C, additional turnover is needed to overcome the problem.

e. Finished Koji Twenty hours after the second turnover, the elongation of the mycelia will stop with the appearance of spores. This is the time to terminate the soya koji production process. Holding the koji mixture overnight will evaporate off additional moisture. The soya koji is ready and is called the finished koji. It took three evenings starting from the beginning step to the end, and therefore the finished koji is also called 4-day-koji. Visibly, the surface of the koji is covered with gray mycelia without spores on the surface. However, there are many yellowish green spores inside, with the mycelia extended into the soy beans. The better koji should be drier, without offensive odor. The moisture content is about 26%. The weight of finished koji should be the same as the original weight of the raw materials.

The purpose of making soya koji is to produce strong protease enzymes without offensive odor or taste, with minimum contamination by foreign microorganisms (especially *Bacillus subtilis*), and minimal loss in raw materials. The soya koji making process should be limited to 45 hr to achieve strong protease activity. It is known that the protease activity can be increased when the soya koji is produced at about 25°C (In general, koji produced at higher temperature has higher saccharifying ability, but lower temperature can produce koji with higher acidic protease activity). However, the optimum growth temperature for the mold is about 33°C. In the past, koji production took 72 hr (4-day-koji). In order to reduce contamination from foreign microorganism, the soya koji making time is thus reduced to 45 hours (3-day-koji) at an increased temperature of 30°C.

3. Mechanical Soya Koji–Making Process

In the mechanical soya koji–making process, if the temperature, oxygen, and moisture are suitable, there is no need for manual operations to achieve good soya koji. There are many ways the koji can be made mechanically (10). One way is to have forced aseptic air on the surface or bottom of the 30-cm-thick koji mixture to maintain temperature and moisture. Another method is to have air with suitable relative humidity and temperature circulating around the thinner koji mixture. In the mechanical process, the chamber is slowly rotating to achieve the mixing effect. Or aseptic air is injected to maintain temperature and moisture. Any of these methods can produce koji with strong enzymatic activity. When the mash is prepared, the enzymes will then fully hydrolyze and ferment to produce a good quality product. For example, to produce the koji starter, start at 28°C on day 1. After 4 hours, most of the spores complete germination with temperature increased to 30°C, encouraging the development of the mold. At the 8th and 16th hours of day 2, start the mechanical mixing. Thereafter, the temperature is maintained at 25°C. The koji making is completed in a total of 48 hr.

Soya koji made on starter trays is labor-intensive and of inconsistent quality. Nowadays, most manufacturers use the mechanical ventilation process. It saves labor and results

in uniform soya koji with strong enzymatic activity. Mechanical ventilation can be divided into forced ventilation and surface ventilation. The former is again divided into forced ventilation on beds and rotating drum model. Forced ventilation on beds is used most often. In the koji room, the koji mixture is evenly piled up to 20–40 cm thick on perforated trays. Air with adjusted temperature and moisture content is forced to the koji mixture through the bottom, encouraging the growth of the mold mycelia and also adjusting the temperature of the koji mixture. Part of the air coming out through the koji mixture to the chamber is vented out. This amount of air is replenished with temperature-controlled fresh air through the bottom of the koji mixture and is circulated. The air going into the chamber is washed with fixed-temperature clean water, providing clean air with consistent temperature and moisture content. The purpose of this forced air ventilation is to (a) provide the mold with enough oxygen, (b) remove the carbon dioxide produced by the mold through respiration, (c) maintain optimum temperature most suitable for mold spore germination and mycelial growth, and (d) inhibit the temperature increase due to respiration heat caused by mycelial growth, thus increasing the enzymatic activity of the koji.

4. Utilization of Components in Raw Material During Soya Koji Making

The carbohydrates in the raw material reduce significantly, approximately by one-third to one-quarter, mainly the starches and other hexoses. These components serve as nutrient (or energy) source and are utilized through hydrolysis during the reproduction of the molds. Part of these carbohydrates is converted to alcohols or carbon dioxide, and eventually evaporated. The proteins are hydrolyzed into smaller molecules, with essentially no change in total nitrogen. Also, during mold reproduction, small amounts of organic acids such as lactic acid, succinic acid, gluconic acid, fumaric acid, and kojic acid are produced. Therefore, the soy koji produced is slightly acidic.

E. FERMENTATION

1. Formulation of Raw Materials

In general, equal amounts of defatted soybeans and wheat are used in the manufacturing of soy koji. The volume of brine at Baume 19–20° (22.5–23.7% sodium chloride, average 23.1%) is 1.2 times the total volume of soybeans and wheat—i.e., approximately the sum of weight of soybean (divided by specific gravity 0.6) and weight of wheat (divided by specific gravity 0.75). This is called "12 water." Brine added at 1.1, 1.3, and 1.4 times are called "11 water," "13 water," and "14 water," respectively. For example, soya koji mixture made from defatted soybean (1000 kg) + wheat (1000 kg) + starter koji (2000 g) mixed with 3600 liters of brine at the time of pasting is equivalent to "12 water." Assuming the utilization rate of raw materials is 82%, 4011 liters of raw soy sauce at 17% salt will be recovered {[(3600 + 299.9) × 12.6%] divided by 17% × 82%}.

2. Mixing of Brine and Soya Koji Mixture (Mashing or Fill-in-the-Crock)

Brine cooled to 0–5°C in the summer can avoid sedimentation and darkening of soy sauce. The mixing container, such as a cement or fiberglass tank, is filled up with brine (Fig. 5). Disintegrated and cooled soya koji mixture is then added. Compressed air is used to stir the mixture. The amount of mashing water (brine) is in the "10 water" to "11 water"

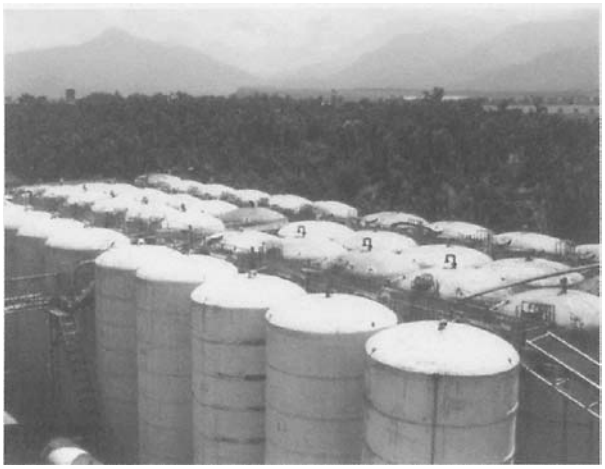

Figure 5 Fermentation tank.

range. Nowadays, when defatted soybeans are used, it is common to use "12 water" to "13 water." Use of lesser amounts of brine will result in better quality soy sauce.

3. Mixing of the Mash

After the mash is mixed for one week, the soya koji will float to the top. It is important to break up this layer with the stirring rod to allow efficient absorption of water. If the breakup is too slow, the part with inadequate mixing of brine and soya koji will generate heat, causing harmful bacteria to develop and produce offensive odor. In the past, when complete mixing was impossible, it was common to punch holes in the floating soya koji with the stirring rod to let the brine cover the soya koji. The current practice is to fully mix the mash with compressed air at the beginning of the scheduled workday. It is very convenient. For example, for the first 7 days, mix the mash every day for 10 min. On the 8th, 10th, 14th day, again mix for 10 min each. On the 20th, 28th, 32nd, 34th, 37th, and 40th day, mix for 7–10 min each. After the 40th day, mix for 10 min every week. When the mixing is too frequent, the mash will become too viscous and affect the fermentation. The mixing should start from every 2 days to every 3 days and is reduced gradually. Temperature of the mash should be at 15°C after 1 month, then the room temperature is increased to 28°C. After half a month, the mash temperature will increase to 28°C and encourage the fermentation. After about 5 months, inject cold air to lower the mash temperature to 15°C. After this period, mixing of the mash depends on the fermentation or maturing condition of the mash. In the summer, when fermentation is vigorous or when the mash is newly mixed, mixing at 3–4 days each is adequate, whereas, mixing should be done every 10 days in the winter months.

Mixing of the mash introduces fresh air to the mixture, allowing the growth of yeast or other beneficial microorganisms and inhibition of the growth of undesirable anaerobic bacteria. At the same time, it helps evaporation of unwanted carbon dioxide and hydrogen sulfide, oxidative coloration of the product, and homogeneous mixing of the mash. In the past, it was known that the better the mixing of the mash, the better the product. Nowadays, with the use of defatted soybeans, the raw materials dissolve easily. Instead,

extensive mixing will induce viscous mash, and reduce the utilization rate, resulting in poor flavor. The rule of thumb is to mix the mash when the white mold does appear.

4. Microorganisms During Fermentation

After mixing, there are different kinds of microorganisms living in the mash responsible for its fermentation and maturation (11). They are discussed below.

a. Molds The molds in the soya koji preparation stage will excrete protease, amylase, and other enzymes. They are responsible for the hydrolysis of the raw materials. These molds cannot survive the high salt concentration and anaerobic condition. They will all die in three months after the mixing of the mash, leaving the enzymes to complete the hydrolysis.

b. Yeasts The yeasts that grow in the mash come from the soya koji and the environment, and they are salt-tolerant. They are composed of two groups: those responsible for the main fermentation and those responsible for the maturation. The former is mainly *Saccharomyces rouxii*. It is a round or oval, bottom-fermentation-type yeast (non–film forming). It is capable of alcoholic fermentation and hydrolysis of various amino acids into their respective alcohols in 22% salt condition, giving the soy sauce its characteristic flavor and odor. At the same time, glutamic acid is converted to succinic acid. During alcoholic fermentation, other organic acids such as acetic, lactic, and succinic acids are also produced, giving the soy sauce a rich flavor and bright color. The maturation yeast is mainly *Torulopsis versatilis*. It grows at the maturation stage of the soy sauce, with production soy sauce odor compounds such as 4-ethylguaiacol. It is not as salt-tolerant as the former, with also weaker alcoholic production capability.

c. Bacteria There are many useful bacteria in the mash. The main ones are lactic bacteria. The most important one during maturation is *Pediococcus halophilus*, which consists of a chain of four cocci and is an anaerobic or slightly aerobic homo-type lactic bacterium. It can utilize the pentoses. It is salt-tolerant and grows in 20% salt solution, it has strong fermentation capability, producing dl-lactic acid. Besides the lactic bacteria, there are *Bacillus subtilis* and *Bacillus mesentericus*, with strong fermentation capability, but essentially no presence of acetic bacteria.

5. The Condition of Maturation in the Mash

The chemical changes during fermentation of the mash are very complex. First, the proteases from the koji or other bacteria hydrolyze the protein in the soybeans and wheat, forming tasty amino acids. The actions of these enzymes are significantly inhibited in the high salt environment. At the same time, hydrolysis is very slow. This is why it takes a long time to make soy sauce. Starch in the wheat is hydrolyzed by the saccharidase into maltose or glucose. This reaction can be completed earlier.

The yeast convert the sugars into alcohol, succinic acid, and other organic acids. The yeast also convert the amino acids into alcohols, providing the characteristic flavor. The sugars are converted by the lactic bacteria into lactic acid, lowering the pH of the mash with flavor stabilization effect.

Bacillus subtilis and *Bacillus mesentericus* also help produce organic acids, amines, and esters, creating a more complex flavor. The lipids in the raw materials are hydrolyzed by esterases into free fatty acids and react with alcohol to form ethyl esters, the so-called soy sauce oil. Besides the amines and sugars, especially the pentoses react to form the melanin pigments. With the aging of the mash, the color turns darker. The formation of melanin is related to oxygen and the mash color darkens from the surface to the bottom.

The soy sauce mash is acted upon by the koji molds, bacteria, and yeasts. It is a complex situation and is not clear which is the most important. All three groups of microorganisms have their own roles. This is quite different from the fermentation of sake, beer, or wine.

In the summertime, with higher temperatures, the yeasts proceed with a strong alcoholic fermentation, producing carbon dioxide with cracking of the mash surface. The mash liquid will rise and this stage is called fermentation.

After a year or so, the soya koji mixture dissolves completely, causing the mash to form a paste. With mixing of the other flavoring materials and salt, soy sauce is produced with its characteristic flavor, mainly 4-ethylguaiacol. For large-scale mixing of the mash, it takes 10 months to complete this digestion and maturation process outdoors in tanks. For small-scale mixing, it takes 1 to 1.5 years.

6. Spoilage of the Mash

Occasionally, when the brine concentration is low, spoilage microorganisms will grow and dominate, hydrolyzing the amino acids to offensive odorous compounds such as phenol, cresol, indole, skatole, methyl mercaptan, and hydrogen sulfide. This phenomenon is called spoilage of the mash and the mash is not suitable for consumption. Usually, lactic acid produced increases significantly without offensive odor. Acid spoilage is a situation in which the significant increase of acid inhibits the hydrolytic reactions.

7. Pressing

In the past, cloth bags of 17 cm (width) × 70 cm (length) bags were used (now nylon bags) to hold the fermented mash for pressing. The filled bags are laid flat in a hydraulic press overnight for gravitational filtration. The pressed bags are then piled on trays for final pressing under pressure. The soy sauce will drip out slowly. More recently, the trend is not to use bags but to use nylon sheets to hold the fermented mash. For each nylon sheet, 12 kg of fermented mash is placed inside. A pile of 330 sheets will permit natural filtration, followed by pressurized filtration (Fig. 6). This method is convenient with high filtration rate. The damage to the nylon sheets is minimal. The liquid coming out from the first filtration is called raw soy sauce (Table 3) and the cake is called raw cake. The raw cake

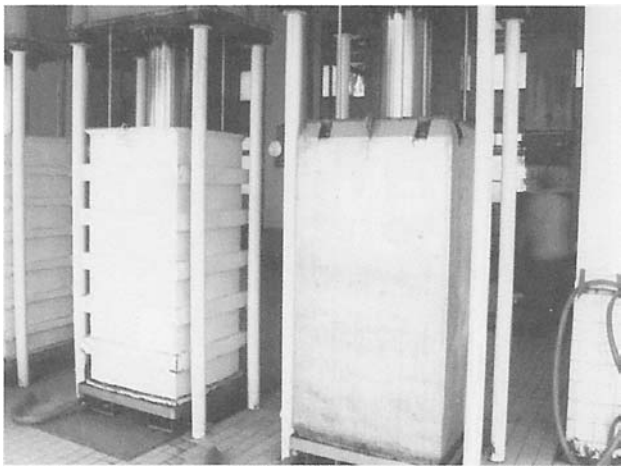

Figure 6 Soy sauce mash presser.

Table 3 General Composition of Raw Soy Sauce (12 Water, %)

Baume	Total nitrogen	Amino nitrogen	Direct reducing sugar	Sodium Chloride	Acidity I	Acidity II	pH	Buffering capacity	Alcohol
23	1.80	0.90	3.3	19	12	11	4.8	0.88	1.2

Source: Ref. 7.

still contains a considerable amount of soy sauce. It can be redissolved in water or dilute brine, or what is called second wash. The mash is pressed again. The filtrate is then called secondary soy sauce or secondary wash. The cake is called soy mash cake. Mixing of the raw soy sauce and the secondary soy sauce in a proper proportion will provide a soy sauce that meets the standard required. In recent years, the manufacturers of above-standard soy sauce (in Taiwan) don't even recover the secondary soy sauce. In general, 1 kiloliter of soy sauce mash will produce 0.6 to 0.8 kiloliter of raw sauce.

8. Pasteurization and Processing

After the raw soy sauce and the secondary soy sauce are mixed, the pasteurization of this "raw" soy sauce through either plate or tube heat exchanger to 87–89°C to inactivate the molds, yeasts, and bacteria is a very important step.

a. Purpose of Pasteurization The purpose of pasteurization is to (a) "mature" the soy sauce flavor by removing the undesirable raw soy sauce flavor and induce the appetizing flavors such as aldehydes and acetals; (b) kill off all the living microorganisms in the fermentation process to guarantee the quality; (c) inactivate all the enzymes soluble in the raw soy sauce and avoid changes in quality; (d) precipitate any residues; (e) improve and brighten the color by increasing the melanin, and (f) solubilize the antifungal agents that are soluble only after exposing the soy sauce to high temperature.

b. Temperature of the Heating Process The liquid soy sauce should be heated to 75°C with holding for 30–40 min, or just heated to 80°C. After heating, the soy sauce should be transferred to another container for natural cooling.

c. Equipment for Heating In the past, heating was conduced directly in heating kettles or double-jacketed steam kettles. In the direct heating process, the kettle and subsequently the soy sauce will be exposed directly to the heat source. Overheating will produce burnt odor. It is better to use the indirectly heated double-jacketed steam kettle, with continuous stirring of the soy sauce and at the same time skimming off of the foamy foreign matter. In recent years, all the above-average manufacturers (in Taiwan) use the continuous multi-tube heater or heat exchanger. This method is easy to operate with less loss of the volatile odors, and is more desirable. Antifungal compounds (preservatives) should be added at this heating stage.

d. Addition of Additives In general, additives are added at the heating step. If raw soy sauce is the only ingredient, then only antifungal compounds are added at this stage. In practice, when the secondary soy sauce is the major ingredient (with the addition of a large quantity of the cake components), addition of additives including bulking agents is needed to standardize the product.

The common additives include sweeteners (such as sugar, refined molasses, licorice extract, artificial sweeteners), umami (meaty flavor) enhancers (such as amino acid solution [protein hydrolysate], sodium-L-glutamate, and 5′-ribonucleotides), succinic acid, or its disodium salt. Proper addition of these additives can improve the taste of soy sauce. Protein

hydrolysate is made by acid hydrolysis of plant protein, including soy protein. Hydrochloric acid at 18% concentration is added to the raw plant protein at a ratio of 1.5 times its weight. The mixture is boiled for 10 hrs, to hydrolyze the proteins. It is then neutralized by adding sodium carbonate powder, adjust in the pH to 5.0–5.2. This neutralized liquid is filtered to remove the black melanin. The protein hydrolysate is orange-brown in color with strong umami-taste and characteristic odor. It is used commonly as umami enhancer in soy sauce; the amount can be the same as the amount of real soy sauce. Protein hydrolysate can also be added at the heating/pasteurization stage, or it is added to the mash before it is heated and pressed.

Sugar is added at 1–2%. Caramel is added to enhance the color intensity. It is made by heating sucrose or glucose at about 141°C. It is a dehydro-polymer of sucrose or glucose. It is a black tarlike oily substance and very soluble in water. It is used commonly in enhancing the reddish-brown color of sauces or colas. Caramel is added to soy sauce at 0.3–0.5%. Japanese Mirin can also be added to the finished soy sauce at 0.5–1% to improve the odor. Alcohol can also be added at 1%.

e. Sedimentation Heating causes the production of pasteurization lees. This sediment is a solidified product of some soluble proteins that react with phosphates and other inorganic compounds during heating. The sediment will separate after the pasteurized soy sauce is held cool for 1 week. The supernatant is recovered. Commercial sedimentation agents are also available.

f. Filtration Filtration is applied when the removal of sediment is difficult and when clear soy sauce is desirable. Filtration can be conducted through supplemental filtration units. Celite (made by heating diatomous earth and subsequently refined) is a commonly used filter aid, and it is very efficient. After filtration, the soy sauce is clear and will not show cloud sediments.

9. Packaging and Quality

The clear, pasteurized soy sauce is filled into clean, suitable containers such as barrels, glass bottles, cans, or polyethylene (PE) drums, or PET (polyethylene terephthalate) bottles. They are sealed and stored or sold. In recent years, there has been no necessity to add undesirable additives in the manufacture of PET bottles. They have the benefits of safety, resistance to moisture loss, good coloring capacity, resistance to moisture and oxygen transfer, crash resistance from drops, minimal odor absorption, transparency, and light weight. Therefore, they are used commonly. Thicker soy sauce can be stored more safely.

In the summertime, molds may grow on the surface of soy sauce; therefore, use of antifungal agents (preservatives) is warranted. Mold is among the three enemies of soy sauce. The other two are bitterness and cloudiness.

Good soy sauce products should have clear red color; possess characteristic odor and flavor, be absent of burnt flavor and offensive odor, sour, and astringent bitter taste, and have proper composition. Table 4 is a comparison of the composition of three grades of soy sauce in Taiwan, R.O.C. (12).

Besides, in the production of protein hydrolysate, there is a carcinogenic by-product of 3-chloro-1,2-propanediol (MCP). Therefore, in the production of chemical or combined soy sauce, the addition of protein hydrolysate should be limited to less than 0.1 ppm of MCP in the final product.

a. Soy Sauce Molds Although mold is not a true fungus (mycelium type), the real situation is the film-forming yeast. This is mainly *Saccharomyces rouxii* (formerly called *Zygosaccharomyces salsu*, *Zygosaccharomyces japonicus*). This yeast is salt-tolerant. When it grows extensively, it will damage the appearance of the soy sauce and digest the sugars

Table 4 Comparison of Soy Sauce of Different Grades

Composition	Grade A	Grade B	Grade C
Nature of product	Color, odor, and flavor of characteristic, fermented soy sauce, absence of undesirable odor or taste	Color, odor, and taste of typical soy sauce, absence of undesirable odor and taste	Good color, taste, and odor, absence of undesirable odor and taste
Total nitrogen (g/100 mL)	1.4 or above	1.1 or above	0.8 or above
Amino nitrogen (g/100 mL)	0.56 or above	0.44 or above	0.32 or above
Total solids less salt (%)	13 or above	10 or above	7 or above
Foreign matter	None	None	None
Contents	Same as identified	Same as identified	Same as identified

Note: Levulic acid content in fermented soy sauce is less than 0.1%.
Source: Courtesy of the National Bureau of Standards, Ministry of Economic Affairs, R.O.C.

and amino acids with deterioration in flavor. The odor will also be damaged with loss of commercial value.

b. Antifungal Agents (Preservatives) and Their Application In order to completely inhibit the growth of molds and protect the quality of soy sauce, the proper application of antifungal agents (synthetic preservatives) is, therefore, necessary (12). The most common antifungal agent (in Taiwan, R.O.C.) is butyl-p-hydroxybenzoate. The amount is only 0.005% of the soy sauce, or 50 ppm, to be effective. However, this white-powdered compound is water insoluble. It can be dissolved first in 5% sodium hydroxide solution (4 times the volume), alcohol, or propylene glycol, and then added to the soy sauce when it is heated to 75–80°C with adequate agitation. It will then properly dissolve in the soy sauce with the proper effect. This antifungal agent can also be applied effectively and directly, without previous dissolution, to the soy sauce with thorough mixing and maintaining the temperature of the soy sauce at 80°C for 5 min or longer. Thorough mixing with 0.05% sodium benzoate at 70°C or above is also effective. Recently addition of *Saccharomyces rouxii* in the fermenting mash to increase the alcohol production has also been practiced.

10. By-Product Utilization

a. Soy Sauce Filter Cake Prepare a 20 liter solution with 1.5–2.0 kg salt and 20 mL glacial acetic acid. Add 10 kg of soy sauce filter cake and let it dissolve. Let it sit overnight and then press the mixture to recover the secondary soy sauce. This secondary soy sauce has less taste and odor compared to raw soy sauce. Therefore, it cannot be marketed directly as soy sauce. It has to be mixed with raw soy sauce or amino acid liquid (protein hydrolysate), sweeteners, and caramel in proper ratio to produce the properly flavored, lower-grade product.

In the past, soy bean filter cake was discarded. Nowadays, it is used as a substrate for soy koji, or a substitute for acid digestion of soybean targeted for animal feed.

b. Soy Sauce Oil The soy sauce oil obtained from pressing of the fermented mash comes mainly from the denatured oil in the soybean. It has the brown appearance of soy sauce and is very viscous. The neutral oil is mainly ether esters. It is used as substrate for making soap or as deodorant in the production of amino acid liquid.

c. Pasteurized Sediment The pasteurized sediment is used for pickling cucumbers or other vegetables.

11. Accelerated Fermentation Methods

Because the traditional fermentation method takes a long time to complete the process, fast fermentation methods are being developed and used.

a. High-Temperature Fermentation Heat up the mash to 28–30°C to allow increase in yeast growth followed by aggressive fermentation. Reaction for the lactic acid production is accelerated. Also, there is an increase in enzymatic reactions to hydrolyze the raw materials. This method takes about 3 months to complete the fermentation.

b. Digestion Process Heat up the mash to 40–45°C. Enzymatic hydrolysis of the proteins and starch is accelerated. In about 2 weeks, the mash is dissolved fairly completely. Then, lower the temperature to 30°C with addition of 10% starter (seed) culture. Hold for 1.5 months to allow extensive fermentation before pressing. This product has rich flavor and color but has a slight residual odor due to high temperature fermentation. When the soya koji is mixed with brine at Baume 12° and held at 40–45°C to conduct the so-called lower salt digestion process, the soya koji will be dissolved in about 1 week. When salt is added to adjust the salt content with lowering of the temperature to about 30°C, addition of the starter (seed) culture will accelerate the fermentation with production of product with less undesirable odor due to high temperature fermentation. Even with the lower salt content, the higher-temperature mash will not spoil.

c. Electrically Accelerated Fermentation Pass alternate current of 3–8 volts and 80–280 amps with a carbon electrode through the mash. With suitable holding temperature, the fermentation can be completed in about 3 months. This product has very good flavor but carries an odor of high-temperature fermentation.

d. Mixed Piling Method Mix the soya koji with "7 water" brine. The hard soya koji will dissolve fairly completely at 40°C in about 2 weeks. Add "5–6 water" brine to form the regular soft mash. After the addition of starter (seed) culture, the fermentation can be completed in about 2 months. This product has very good odor, flavor, and color, with rich umami and sweet taste. It does not require complicated technology, and it is not labor intensive. It has a high nitrogen utilization rate.

e. Added Yeast Method In the warmed brewing mash, mother (starter) mash is a result of the culturing of yeast and lactic acid bacteria. Addition of this kind of yeast at 5% will accelerate the fermentation with production of an excellent soy sauce. Also, the utilization rate is high, similar to the result of enriched mold starter method.

III. CHEMISTRY AND BIOCHEMISTRY IN SOY SAUCE MANUFACTURING

A. Major Chemical Composition of Soy Sauce

Free amino acids and peptides may be derived from the enzymatic digestion of soy and wheat protein via proteolytic enzymes. Sugars are the degraded products from carbohydrate materials by the amylolytic enzymes. Koji is a source of these proteolytic and amylolytic enzymes. Macerating enzymes are very important for the solid-liquid separation. Free sugars—arabinose, mannose, galactose, xylose—are the digested products from the middle lamella materials by the macerating enzymes. In addition to the enzymatic hydrolytic reaction, some enzyme-derived aroma syntheses generate key volatiles for typical soy sauce flavor. The fermentation process both improves the organoleptic quality of legumes and enhances their nutritional quality (13).

Chemical reactions involving protein denaturation and starch gelatinization are found to enhance the enzymatic digestion. Under the heat treatments in roasting, steaming, and

sterilization steps, Maillard browning reaction, thermal aroma generation, and caramelization may occur. During severe heat treatments, tryptophan and glutamic acid are modified.

Sophisticated chemical and biochemical reactions occur during the whole soy sauce processing. The major raw materials for soy sauce processing are soybeans (defatted soy flour), wheat grain, salt, and water (14). As raw materials, soy protein, wheat protein, carbohydrate (starch, cellulose, hemicellulose, lignin, pectic substances) are converted into small molecules or derivatives that contribute to the sensational aroma and taste of a good-quality soy sauce. Lipids from both soybeans and wheat grains are degraded as well during soy sauce processing. In general, soy sauce contains 1.0–1.65% total nitrogen (w/v), 2–5% reducing sugars, 1–2% organic acids, 2.0–2.5% ethanol, and 17–19% sodium chloride (w/v). About 45% of the nitrogen is found in simple peptides, and 45% in amino acids (9).

Among the free amino acids, glutamic and aspartic acids are the major amino acids presented in the final products. A total of 18 amino acids are present in the fermented soy sauce. A representative Japanese fermented sauce contains the following sugars %(w/v): arabinose, 0.08%; glucose, 2.05%; mannose, 0.06%; galactose, 0.17%; xylose, 0.06%; disaccharide, 0.65%; polysaccharide, 1.15%; unidentified sugar, 0.23%; total sugar, 4.45% (as glucose) (15). Organic acids are some of the most important constituents of soy sauce. The acids predominant in the moromi in the early brewing stages are citric acid and malic acids, which then disappear during the lactic acid fermentation (16). Organic acids in soy sauce products are acetic acid, 0.16%; citric acid, 0.04%; formic acid, 0.02%; lactic acid, 0.68%; succinic acid, 0.05%; total 0.95%. Only very small amounts of free sugars and 5' nucleotides in the soy sauce products were detected. Investigators postulated that these two categories of compounds contribute only little to the flavor of soy sauce.

Biogenic amines, such as tyramine and beta-phenylethylamine, have been proposed as the initiators of hypertensive crisis in certain patients and of dietary-induced migraine. Another amine, histamine, has been implicated as the causative agent in several outbreaks of food poisoning. Soy sauce contains histamine along with other biogenic amines. Microorganisms possessing the enzyme histidine decarboxylase, which converts histidine to histamine, are responsible for the formation of histamine in soy sauce (17).

Chemical and biochemical reaction during soy sauce processing will be discussed in the following sections in detail.

B. Protein Denaturation and Starch Gelatinization

In koji preparation, soybeans are soaked in water and then cooked under pressure in a retort or a continuous cooker. The wheat is heated in a continuous roaster with hot air at 150°C for 30–45 sec at atmospheric pressure. In the making of regular soy sauce, the cooked soybean (or defatted soy flakes) are mixedwith an equal amount of roasted wheat and then inoculated with 0.1–0.2% of starter mold (*Aspergillus oryzae* or *Aspergillus sojae*). Extrusion cooking at elevated temperature of cereal raw materials results in a significant chemical and physical modification of carbohydrate (18) and protein (19) components. The results indicate that protein denaturation and starch gelatinization are crucial chemical reaction in soy sauce processing. Recently, a high-temperature short-time food extruder was applied to denature the protein in soybean and wheat grain. It was concluded that fermentation substrates subjected to extrusion pretreatment were better utilized, and at reduced fermentation cost.

Mycelial propagation, activity of various enzymes, and some chemical components were examined during the preparation of soy sauce koji with *Aspergillus oryzae* or *A. sojae* on extruded and traditional raw materials. Results showed that *A. oryzae* grew better on the extruded substrate than on the traditional raw material after 3 days of cultivation, whereas no difference in mycelial propagation was noted when *A. sojae* was used as the starter.

Higher activities of protease, amylase, alpha-galactosidase, glutaminase, and lipase were noted in koji prepared with the extruded substrate than that prepared with the traditional substrate (20).

The chemical and physical properties of protein were modified either in the conventional steaming and roasting process or the innovative extrusion step. The digestibility of soybean and wheat proteins by proteolytic enzymes is markedly improved by the heat treatments. Native protein molecules are unfolded and subjected to attack by the proteases present in koji. The growth and enzyme production by *Aspergillus oryzae* were greater in the extruded substrates than in traditional substrates (21). Product expansion ratio, bulk density, protease susceptibility, α-amylase and β-amylase susceptibility increased in the extruded substrates as compared with that of the traditional substrates. The dosage of koji preparation in soybean sauce fermentation could be reduced by using co-extruded wheat and soybean flour (22).

During the aging of the soy sauce mash for 180 days, the contents of total nitrogen, amino nitrogen, free amino acids, and reducing sugars and the protein utilization rate were higher in soy sauce prepared with extruded raw material than with traditional raw material. No marked difference in pH was noted between the two types of soy sauce prepared. However, a much higher increase in the intensity of brown color was noted in soy sauce prepared with extruded substrate than that prepared with traditional substrate (23).

C. Chemical Modification of Amino Acids

Too much heat treatment decreases the extent of proteolysis by causing other changes in the proteins. α- and ε-amino groups may be modified by the Maillard reaction. The modification of the lysine and arginine residues on the protein molecules may reduce the digestability of koji protease. Some of the koji protease attacks the side chain of basic amino acid residues, such as lysine and arginine. Glutamine is chemically changed to pyroglutamic acid, which has no taste (24). During severe heat treatments, lysine can react with glutaminyl and asparaginyl residues to give isopeptide bonds, that is ε-(γ-glutamyl)lysine and ε-(β-aspartyl) lysine, respectively (25). The N-terminal glutamine residues of the proteins was dehydrated into pyrolidone-carboxylic acid under severe heat treatment. Tryptophan residues are transformed into mutagens during pyrolysis (26).

The occurrence of tetrahydro-beta-carboline-3-carboxylic acids (THbetaC-3-COOHs) in soy sauces was investigated. Spectral and chromatographic data showed the occurrence of 1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (THCA) and 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid (MTCA) in foodstuffs. The content of THbetaC-3-COOHs given as a sum of both THCA and MTCA in soy sauce was 94–517 mg/L. Experiments in which foodstuffs were spiked with formaldehyde and acetaldehyde proved the chemical formation of THCA and MTCA, respectively. The exogenous intake of these substances during the human ingestion of foods may be partially responsible for the reported endogenous presence of THbetaC-3-COOHs in human biological tissues and fluids (27). THCC derivatives derived from Pictet-Spengler condensation of tryptophan with alpha-oxo acids has been detected (28).

Soy sauce was found to show antiplatelet activity. The active components in soy sauce with inhibitory effects on the aggregation of human platelets were identified as 1-methyl-1,2,3,4-tetrahydro-beta-carboline and 1-methyl-beta-carboline on the basis of EI-MS, (1)H NMR, diode array, and fluorescence spectra. The quantitative HPLC analysis revealed that significant amounts of both antiplatelet compounds were uniformly contained in commercially available soy sauce. From these results, soy sauce may be referred to as functional

seasoning containing alkaloidal components with a potent preventive effect on thrombus formation (29).

D. Oxygen-Dependent Browning of Soy Sauce

It is well known that soy sauce darkens during storage in contact with atmospheric oxygen. Kato (30) suggested that 3-deoxyglucosone (3-DG) was an active precursor in a sugar-amine browning reaction, and Okuhara et al. (31) reported the participation of peptides in the browing of soy sauce. Motai and Inouue (32) isolated melanoidin from soy sauce and examined its oxidative polymerization and physicochemical properties. Although caramel is used as an additive to adjust the color of soy sauce products, the most preferable color of soy sauce is red-brown soon after pasteurization. The browning of soy sauce is considered to be due to the amino compounds, peptides, sugars, and intermediates. There is little browning of soy sauce without oxygen, but that browning is very significant (about 10 times more) in the presence of oxygen (33).

Amadori compounds composed of a neutral amino acid and glucose have been isolated and characterized from soy sauce. These Amadori compounds were demonstrated to be important in oxidative browning of soy sauce. Oxygen decomposes Amadori compounds to produce glucosome and liberate amino acid. Dicarbonyl compounds derived from these Amadori compounds may polymerize to form melatonin. The browning of all the Amadori compounds increased very significantly when oxygen and iron were present. Okuhara et al. (34) suggested the significant positive correlation between initial color [amount of melanoidin] and oxidative browning of soy sauce. Hashiba (35) reported the polymerization and the change of color tone of melanoidin on oxidation. A high proportion of the iron in soy sauce was suggested to be present in high molecular state and could not be removed by dialysis. A large part of iron is bound to melanoidin in soy sauce. Moromi samples of traditional Thai soy sauce were used to investigate the browning and chemical changes related to the Maillard reaction. The browning rate was high at the first 3 days and declined in the later stage, while the accumulation of 5-hydroxy-2-furaldehyde, which is the Maillard reaction intermediate, increased linearly. The results suggested that the browning is enhanced with the proper size of proteins and peptides (36). Table 5 lists the chemical reactions that occur during manufacture of fermented soy sauce.

Table 5 Chemical Reactions Occurring During Manufacture of Fermented Soy **Sauce**

Reactions	Steps	Raw material	Function
Denaturation	Steaming Roasting	Soy protein Wheat protein	Increase digestivity
Gelatinization	Steaming Roasting	Starch	Increase digestivity
Maillard	Steaming Roasting Sterilization	Amino acid and reducing sugars	Color development
Dehydration	Steaming Roasting Sterilization	Glutamine	Pyroglutamic acid
Pyrolysis	Steaming Roasting Sterilization	Tryptophan	β-carboline

E. Microflora in Koji and Moromi

There are several contaminating microorganisms in koji, such as *Micrococcus*, *Streptococcus*, *Lactobacillus*, and *Bacillus*. However, these contaminants are not resistant to the high concentration of salt. Ishigami and Ishikawa (37) isolated some characteristic yellow-green *Aspergillus* strains, which produced prominently echinulate conidia and smooth-walled codiophores, from koji used in the production of soy sauce. They reported the *Aspergillus* strains to be a new species, *Aspergillus sojae*.

The second step in making fermented soy sauce is brine fermentation. It utilizes the lactic bacterium, *Pediococcus halophilus* and the yeasts *Zygosaccharomyces rouxii* and *Candida species*. *Aspergillus sojae* cannot grow in a pH over 6.0 in the presence of 18% salt (w/v). The specially selected *Pediococcus halophilus* is cultured and added to the moromi mash. It is necessary to keep the fermenting mixture at 15°C for the first month, allowing the pH of the mash to decrease slowly from 6.5 to 5.0. The cultures of *Zygosaccharomyces rouxii* and *Candida* species are added as a starter (38). *Candida* species such as *C. versatilis*, *C. etchellsii*, and the like grow at an early stage, but growth is much slower than that of *Zygosaccharomyces rouxii*, because *Zygosaccharomyces rouxii* is more anaerobic than the *Candida* species. The temperature of the moromi is allowed to rise to nearly 28°C until vigorous alcoholic fermentation starts.

The lactic acid bacterium *Tetragenococcus halophila* is the dominant species in Indonesian soy mash. *Tetragenococcus halophila* growing in continuous and retention cultures under defined glucose-limited conditions showed a switch from homolactic (only lactate produced) to mixed-acid fermentation (two formate, one acetate and one ethanol formed per glucose) at low growth rates (39). Chinese- and Japanese-type fermented soy sauces are made of different plant materials. The lactic acid bacterium *Tetragenococcus halophila* is present and grows in both types. In the Chinese type, almost all isolates utilized L-arabinose, whereas in the Japanese type only 40% of the isolates did. Also, the population in the Japanese type was more heterogeneous regarding substrate utilization. Random amplified polymorphic DNA analysis revealed that the heterogeneous population at the Japanese-type industrial manufacturer was derived from only three strains at maximum (40).

Soy sauce was fermented in a column-type reactor with immobilized whole cells of *Pediococcus halophilus*, *Saccharomyces rouxii*, and *Torulopsis versatilis* entrapped in Ca alginate gels. Lactic acid and alcohol fermentation in a feed solution proceeded faster for an enzymatic hydrolyzate of koji and defatted soybean meal than did a complex fermentation of soy sauce mash (moromi) in slurry. The refined fermentation products obtained with the immobilized microbial cells were similar to conventional soy sauce in organic acid and aroma composition and possessed an acceptable taste and flavor (41). The optimum conditions for continuous alcohol fermentation of soy sauce with immobilized *Zygosaccharomyces rouxii* cells were investigated using an airlift reactor. The optimum pH and temperature of the fermentation were 4.5–5.5 and 25–27.5°C, respectively. The products fermented by supplying air (0.02 ppm) had higher content of aroma components than that by supplying only nitrogen gas, and the aroma of the former products was similar to that of conventional soy sauce (42).

F. Enzyme System in Soy Sauce Processing

The enzymes—including maceratings, amylolytic, and proteolytic enzymes—of koji are used in the preparation of soy sauce. Filamentous fungi are prodigious producers of enzymes used in the production of soy sauce (43). Table 6 is a summary of enzymes involved in the manufacturing of sauce.

Table 6 Biochemical Reactions Occurred During Fermented Soy Sauce Manufacturing

Enzymes	Major products
Acidic protease	Peptide
Alkaline protease	Peptide
Aminopeptidase	Amino acid
Glutaminase	Glutamic acid
Amylase	Reducing sugars
β-amylase	
α-amylase	
Cellulase	Neutral sugars
Hemicellulase	Reducing sugars
Xylase	
Arabinase	
Galactanase	
Pectin lyase	
Polygalacturonase	
β-glucodidase	
Lipase	Free fatty acids

1. Macerating and Amylolytic Enzymes

Macerating enzymes such as β-glucanases (cellulase C1, CM cellulase, β-glucanase, β-1,3-glucanase), pectinases (pectin lyase, polygalacturonase), hemicellulases (xylanase, arabanase, galatanase) have been characterized as plant tissue degradation enzymes from *Aspergillus oryzae* in soy bean koji (44). Koji prepared by solid fermentation is superior to that of the liquid culture because of the existence of macerating activity. The macerating enzymes enhance the contact btween the proteolytic enzymes and the proteins through the disruption of the cell wall structure in the soybeans and cereals.

A genomic gene encoding a polygalacturonase from *Aspergillus oryzae*, used in soy sauce production, was cloned and sequenced. The structural gene comprises 1227 bp coding for 363 amino acids with a putative prepropeptide of 28 amino acids and the open reading frame is disrupted by two short introns of 57 bp and 81 bp. The deduced amino acid sequence of the mature protein showed 63%, 63%, 63%, and 64% homology with those of *Aspergillus niger* polygalacturonase I, *Aspergillus niger* polygalacturonase II, *Aspergillus tubingensis* polygalacturonase II, and Cochliobolus carbonum polygalacturonase, respectively. There is, however, little homology among fungal, plant, and bacterial polygalacturonases (45). Beta-xylosidase secreted by the shoyu koji mold, *Aspergillus oryzae*, is the key enzyme responsible for browning of soy sauce. To investigate the role of beta-xylosidase in the brown color formation, a major beta-xylosidase, XylA, and its encoding gene were characterized. Beta-Xylosidase XylA was purified to homogeneity from culture filtrates of *A. oryzae* KBN616. The optimum pH and temperature of the enzyme were found to be 4.0 and 60°C, respectively, and the molecular mass was estimated to be 110 kDa. The xylA gene comprises 2397 bp with no introns and encodes a protein consisting of 798 amino acids (86,475 Da) with 14 potential N-glycosylation sites. The deduced amino acid sequence shows high similarity to Aspergillus nidulans XlnD (70%), *Aspergillus niger* XlnD (64%),

and *Trichoderma reesei* BxII (63%). The xylA gene was overexpressed under control of the strong and constitutive *A. oryzae* TEF1 promoter (46).

2. Proteases

It has been shown that the proteases of *Aspergillus oryzae* or *Aspergillus sojae* include seven kinds of proteases with four different optimum pH values. Of these proteases, alkaline protease has been studied in most detail (47). It is a serine enzyme and is active in a broad pH range (between 6 and 11). Neutral proteases are zinc proteases. Neutral proteases have specificities that are common to the metal proteases of microorganisms. The proteases described above are all endotypes that hydrolyze proteins only into peptides. Free amino acids are not liberated substantially by these proteases. The size of majority of the peptides in soy sauce corresponded to oligopeptides having 3.5 amino acid residues on average. Three glycodipeptides and eight dipeptides were isolated and characterized as the major neutral peptide components in soy sauce (48). Four dipeptides and sugar derivatives of ten dipeptides and two tripeptides were isolated and characterized as the major acidic peptides in soy sauce. However, it was difficult to anticipate any direct contribution of these peptides to the flavor construction in soy sauce on the basis of their contents and taste intensities (49). Recently a novel salt-tolerant protease produced by *Aspergillus* sp. FC-10 was purified to homogeneity. This protease demonstrated an optimum pH range of 7.0–9.0 for activity, with a stable pH range of 5.0–9.0. The optimum process temperature at pH 7.0 was 65°C. The enzyme has a molecular mass of 28 kDa and was deduced as a monomer with an isoelectric point of 3.75. Based on the N-terminal amino acid sequence and amino acid composition, this purified protease should be classified as a member of the deuterolysin family (50).

Koji contains various kinds of exo-type peptidases that liberate the amino acids from the carboxy or amino terminal of peptides successively. At least four kinds of carboxypeptidases and seven kinds of aminopeptidases have been separated from koji (51). All of the aminopeptidases have a high specificity for the amino terminal leucine. Among the endotype peptidases, aminopeptidase II and acid carboxypeptidase IV were especially significant in the hydrolysis of peptides produced by the proteases.

3. Glutaminase

Green vegetable proteins such as soybeans and wheat contain quite a lot of glutamines instead of glutamic acid, glutaminase in koji deserves special mention. Glutaminases are used in the hydrolysis of vegetable proteins to produce L-glutamic acid, which imparts a "umami" taste in soy sauce and improves the flavor (52). Production of glutaminase by yellow-green aspergilli is much larger in a solid culture than in a liquid one. Koji is the most adequate enzyme source for the hydrolysis of cereal proteins because it has a high content of glutaminase as well as the composite peptidases. Green glutaminase is fairly sensitive to acidic pH as well as to salt (53), the brine fermentation in soy sauce making is carried out at a lower temperature (15–20°C) for the first month to prevent the loss of glutaminase activity. A glutaminase from *Aspergillus oryzae* was purified and its molecular weight was determined to be 82,091. On the basis of its internal amino acid sequences, the glutaminase gene (gtaA) from *A. oryzae* was isolated and characterized. The gtaA gene had an open reading frame coding for 690 amino acid residues, including a signal peptide of 20 amino acid residues and a mature protein of 670 amino acid residues. In the 5′-flanking region of the gene, there were three putative CreAp binding sequences and one putative AreAp binding sequence. The gtaA structural gene was introduced into A. oryzae NS4 and a marked increase in activity was detected in comparison with the control strain (54).

G. Aroma Compounds Formation

The chemical nature of soy sauce is a very complex one and is gradually formed during the various stages of its preparation and fermentation. The major aroma compounds have been collected by steam distillation under reduced pressure (55). At least 267 compounds were listed as soybean sauce volatiles, including 37 hydrocarbons, 29 alcohols, 40 esters, 15 aldehydes, 3 acetals, 17 ketones, 16 furans, 4 lactones, 4 furanones, 5 pyrones, and pyrazines, 3 thiazoles, 3 terpenes, and 24 acids, and 2 other compounds (56). In most of the earlier studies of soy sauce flavor, steam distillation, extraction with various solvents, acid or base fractionation, and other preparative methods were applied and there was the possibility that flavor components might have changed chemically during treatment (57).

The contents of the flavor components in soy sauce were analyzed recently (58). The methylene chloride extract from a one-year fermented soy sauce was used. The investigators reported that furfuryl alcohol was the first major flavor compound. The next major compounds were found to be 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone (HEMF), 2,3-butanediol, 2-phenyl ethanol, ethyl palmitate, isoamyl alcohol, and ethyl linoleate, in that order. Among them, HEMF was considered to be the most important aroma contributor. In addition to the major components, significant amounts of minor components, such as 4-ethyl guaiacol, 2-phenylethanol, 2,6-dimethylpyrazine, acetoin, acetol, 3-methylthio-1-propanol (methionol), 3-hydroxy-2-methyl-4H-pyran-4-one (matol), butyllactone, vanillin acetate, were characterized as well.

The amount of flavor compounds produced by yeast depends greatly on concentration of sodium chloride in the culture media and soy sauce. Flavor compounds such as isobutyl alcohol, n-butyl alcohol, isoamyl alcohol, 3-(methylthio)1-propanol, and 2-phenylethanol, which are derived from amino acids by the actions of soy sauce yeast, were decreased with an increase of sodium chloride concentration in the fermented broth. However, the production of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF) biosynthesized through the pentose-phosphate cycle reached a maximum at a sodium chloride concentration of 16% (59).

1. Furanone Formation

Among the aroma compounds characterized in good-quality soy sauce, 4H-pyrone and 3(2H)-furanones are of special interest. 4-hydroxy-5-methyl-3(2)-furanone (HMF), as well as 4-hydroxy-2(or5)-ethyl-5(or2)-methyl-3(2)-furanone (HEMF), with a very low odor threshold, has a caramel-like odor and is organoleptically considered to be one of the most important constitutes of the characteristic soy sauce flavor. Interestingly, a very common soy sauce ingredient, licorice (the root of Glycyrrhiza species) was found to contain significant amount of maltol glycoside, licoagroside B (60).

HEMF gives a mild, soy sauce–like aroma. HMF exhibits a heavier note, a cooked beef–like aroma, and also an odor reminiscent of soy sauce. 4-Hydroxy-5-methyl-3(2)-furanone was isolated and characterized from soy sauce for the first time by Nunomura et al. (61). Sasaki et al. (62) reported this compound to be a product of a reaction between aldopentoses and primary amine. Tonsbeek et al. (63) reported that ribose-5-phosphate and pyrrolidone carboxylic acid or taurine, or both, were natural precursors of HMF. The content of HEMF only increased during the later stages of the fermentation and its yield was highest after 60 days. Biosynthesis route of HEMF was claimed to be through the pentose-phosphate cycle by *Zygosaccharomyces rouxii* (64). It is known that pentoses such as arabinose, xylase, and ribose are present in soy sauce. The precursors of HMF in soy sauce can be the sugars and certain amino acids that react together in the Maillard reactions, including the Amadori rearrangement and subsequent cyclization. The quantity of HMF increased remarkably

during the pasteurization of raw soy sauce, which indicates that the intermediates formed during moromi fermentation play an important role in HMF synthesis.

4-Hydroxy-2,5-dimethyl-3(2)-furanone (HDMF) and 4-hydroxy-5-methyl-3(2)-furanone (HMMF) are derived from a Maillard reaction. During thermal treatment of carbohydrates, several α-dicarbonyls are formed (65). The condensation of the carbonyl group of the reducing sugar with the amino compound gives a glycosylamine. Subsequently, this rearranges and dehydrates, via deoxyosone, to various sugar dehydration and degradation products such as furfural and furanone derivatives. When a ketose is involved instead of an aldose sugar, then a ketosylamine is formed that undergoes a Heyns rearrangement to form a 2-amino-2-deoxyaldose (Heyns product). At temperature above 100°C, 1-amino-1-deoxy-2-ketoses undergo 2,3-enolization to give a 1-amino-2,3-enediol from which an amine is eliminated to form a methyl-2,3-dicarbonyl intermediate (66). 1,2-Enolization of the Amadori product will result, after dehydration and deamination, in the formation of a 3-deoxyosone. The 3-deoxyosone is readily converted to the corresponding furfural from a pentose, and 5-methylfurfural from a hexose. Furan derivatives are also formed by the further dehydration of the 1-methyl-2,3-dicarbonyl compounds, yielding 4-hydroxy-5-methyl-3(2H)-furanone or 4-hydroxy-2,5-dimethyl-3(2)-furanone (HDMF) from the pentose and hexose sugars, respectively (67).

2. Pyrazine Formation

Two pyrazines, methylpyrazine and 2,6-dimethylpyrazine were found in the headspace volatiles of soy sauce. The temperature used during the soy sauce processing is not as high as in cooking, therefore fewer pyrazines are found in soy sauce. Alkylpyrazines were formed from a reaction of the thermally degraded nitrogenous substances, NH_3, RNH_2 from proteins, peptides, amino acids, phospholipids, and α-dicarbonyl compounds in food (68). Formation pathways for alkylpyrazines have been proposed by numerous researchers (69). An important route to alkylpyrazines is from α-aminoketones, which are formed in Strecker degradation or from the reaction of α-dicarbonyls with ammonia. Condensation of two aminoketone molecules yields a pyrazine. It is expected that during the thermal treatment of soy sauce products, all these precursors may participate in the reactions leading to the formation of various heterocyclic soy sauce aroma compounds.

Pyrazine may also be synthesized enzymatically during soy sauce fermentation. The first evidence that microorganisms were able to synthesize pyrazines was provided by Kosuge and coworkers (70), who showed that tetramethylpyrazine could be produced by *Bacillus subtilis*. Several other microorganisms able to synthesize different alkylpyrazines have been discovered (71). 2,5-Dimethylpyrazine and tetramethylpyrazine were produced using *Bacillus subtilis* grown in solid substrate conditions using ground soybeans suspended in water. Optimization studies showed that the best way to produce the two above pyrazines involved massive enrichment of the medium with L-threonine and acetoin. In soy sauce fermentation, lactic acid bacteria produce significant amounts of acetaldehyde, 2,3-butanedione, 2,3-pentanedione, and 3-hydroxy-2-butanone. 2-Oxopropanal is proposed to be the precursor of 2,6-dimethylpyrazine (72). Condensation of 2-oxopropanal with ammonia leads to the formation of α-aminoketones, and incorporation between two molecules of α-aminoketones under mild condition results in the formation of 2,6-dimethylpyrazine (73).

3. Ester Formation

Various kinds of esters are present in fermented soy sauce and their aromatic characteristics contribute to the fruity note of the products. Esters in soy sauce are made by the reaction between the organic acids from the first lactic acid fermentation by *Pediococcus halophilus*

and the alcohols from the second alcoholic fermentation by *Zygosaccharomyces rouxii*. *Pediococcus halophilus* converts 1 mol of glucose to 1.71 mol of L-lactic acid, 0.28 mol of acetic acid, and 0.17 mol of formic acid (74). Galactose, arabinose, and xylose are also converted mainly to lactic acid. One mole of citric acid is converted to 0.16 mol of L-lactic acid, 1.86 mol of acetic acid, and 0.597 mol of formic acid by *Pediococcus halophilus*. A *Pediococcus halophilus* strain isolated from soy sauce (moromi) demonstrated enzymatic activity to transform the dicarboxylic L-malic acid to the monocarboxylic L-lactic acid following the malo-lactic fermentation (75). A partially purified enzyme of *Pediococcus halophilus* converted malic acid to lactic acid in the presence of NAD. Its molecular weight was about 115,000 and optimum pH for activity was 5.8. Malic acid was directly and quantitatively decarboxylated to lactic acid and carbon dioxide. In addition to the production of lactic acid and ethanol by their respective fermentation, a remarkable decrease in the concentrations of malic and citric acids has been observed. Similar to that in volatile composition of wine, significant amounts of ethyl lactate and diethyl succinate were characterized in fermented soy sauce. High quantities of lactic and succinic acid developed by yeast or lactic acid bacteria strains are responsible for their ethyl lactate and diethyl succinate accumulation (76).

Fatty acid ethyl esters are obtained from ethanolysis of acylCoA that is formed during fatty acid synthesis or degradation. Ester concentration and relative distribution is governed by yeast strain and fermentation condition (77). Fatty acid ethyl esters may also be attributed to the reactions of free fatty acid and digestion products of soybean lipid with the yeast-synthesized ethanol during the aging process (78).

4. Phenol Formation

The appearance of 4-ethyl phenol, 4-ethylguaiacol, 2-phenyl ethanol (phenyl ethyl alcohol, floral-rosy), through the action of various yeasts, may also be important to the characteristic flavor profile of soy sauce. The most important role of *Candida* species and *Torulopsis* species fermentation is the production of phenolic compounds, such as 4-ethylguaiacol and 4-ethylphenol (79). Investigators proposed that cinnamic acids series compounds, ferulic acid and p-coumaric acids, are the precursors of the two alkylphenols. Wheat and wheat bran incorporated into the koji preparation contribute a series of guaiacol compounds: vanillin, vanillic acid, ferulic acid, and 4-ethyl guaiacol, which are probably derived from lignin and the breakdown of various glycosides during the heat treatment. Nonvolatile components in commercially fermented soy sauce produced from whole soybeans and defatted soybeans were analyzed by reversed phase HPLC (RP-HPLC). Two soy sauce groups were clearly differentiated by soft independent modeling of class analogy (SIMCA), where ferulic acid was identified as one of the key components for the differentiation (80).

5. Alcohol Formation

Lactic acid bacteria metabolize pyruvate anaerobically, yielding varying amounts of carbon dioxide, acetoin, diacetyl, 2,3-butanediol, acetic acid, ethanol, and lactic acid (81). Volatile acyloins (alpha-hydroxy ketones) are obtained by condensing either aldehydes with pyruvate or 2-keto acids with acetaldehyde in a reaction catalyzed by yeast pyruvate decarboxylases (EC 4.1.1.1). Odor qualities and threshold values of 34 acyloins were evaluated, and 23 of them possessed distinct flavor properties. Soy sauce flavors were analyzed: 2-hydroxy-3-pentanone and 3-hydroxy-2-pentanone were identified in soy sauce for the first time. The biocatalytic efficiencies of crude pyruvate decarboxylase preparations from *Zygosaccharomyces bisporus*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, and *Kluyveromyces marxianus* were compared. Product yields comparable to those of conver-

sions with purified pyruvate decarboxylase demonstrated the suitability of crude enzyme extracts as cost-effective biocatalysts in acyloin formation (82).

Leucine degradation seems to involve chain reactions that commence by transamination, producing a ketoisocaproic acid, followed by nonoxidative decarboxylation into 3-methylbutanal, which is then oxidized or reduced to acid or alcohols (83). The formation of the higher alcohols n-propanol, isobutyl alcohol, active amyl alcohol, and isoamyl alcohol has been studied intensively (84). These higher alcohols are derived either from the deamination or transamination of extracellular amino acids (Ehrlich pathway) or directly from amino acid biosynthetic pathways. In the Ehrlich pathway, the uptake and transamination of isoleucine, valine, and leucine results in the formation of α-keto-β-methylvalerate, α-keto-isovalerate, and α-keto-isocaproate, respectively, which are converted into active amyl alcohol, isobutyl alcohol, and isoamyl alcohol, respectively. Recently, it was suggested that the Ehrlich pathway is not the only pathway involved in the catabolism of the branched-chain amino acids (isoleucine, valine, and leucine) (85). 2-Phenyl ethanol is considered to be the common product produced by yeast fermentation. Formation of 2-phenyl ethanol follows a similar pathway. Similar to that of higher alcohol, Strecker degradation product of methionine leads to the formation of methional, and its reduced product methionol are key aroma compounds of soy sauce flavor (86). Both methional and methionol have very low threshold values (2.0×10^{-1} and 3.0×10^{-1} ppb in water, respectively. (See Table 7.)

Table 7 Aroma Compounds Generated During Manufacture of Fermented Soy Sauce

Classes	Compounds	Precursors
Alcohol	Isobutanol	Valine
	n-Butanol	Isoleucine Leucine
	Isoamy alcohol	Methionine
	Acetol	Phenylalanine
	Acetoin	Ferrulic acid
	2,3-Butanediol	
	Furfuryl alcohol	
	Methionol	
	2-Phenyl ethanol	
	Maltol	
	4-Ethyl guaiacol	
Acids	Acetic acid	Pentose
	Lactic acid	Hexose
	Succinic acid	
Furanones	HDMF	Amino acid and reducing sugars
	HEMF	
	HMF	
Pyrazines	methylpyrazine	Threonine
	2,6-dimethylpyrazine	
Esters	Ethyl lactate	Lactic acid,
	Diethyl succinate	Succinic acid,
		Fatty acid
		Ethanol

IV. SANITARY MANAGEMENT

Establish a sanitation management plan and standard. Conduct periodic inspection.

A. Sanitary Management of the Environment

Within the plant territory, maintain the ground free from accumulated water and dust.

Avoid the piling of unneeded equipment and materials, and the multiplication of harmful animals.

The plant structure itself and the equipment should be maintained clean and sanitary, avoiding contamination of the food.

The trenches (wastewater discharge system) should be kept fluent without the accumulation of soil.

The exhaust gas from the amino acid digestion should be treated to remove the acid.

Solid waste such as the residual cakes and liquid waste should also be properly treated. The waste should be classified, with the easily spoiled waste picked up and discarded every day. In the waste placement site, generation of offensive odor or harmful gases, and the multiplication of harmful animals and insects should be avoided. The waste must not contaminate the food and the floor, or get in contact with the food contact surface and the water source.

B. Sanitary Management of the Plant and Its Equipment

The different establishments inside the plant should be kept clean all the time. The roof, ceiling, and walls should not be damaged.

The floor and the liquid and (solid) discharge systems should be functional, without accumulation of waste water.

Sites for raw material treatment, soya koji making, pressing, sterilization and packaging should be washed at the start of the day.

Sites for raw material treatment and soya koji making should also be sterilized or sanitized. Toilets have to be washed and sanitized every day.

Gases generated by the various operations (including koji making) should be efficiently vented outside the plant.

Outer surfaces of illuminators and pipes should be kept clean.

All animals should be kept outside the manufacturing and storage areas.

In areas where raw material treatment, processing, packaging, and storage are conducted, there should be containers for holding the waste, and they should not leak and should be easily sanitized and covered. They have to be picked up and sanitized periodically.

Restricted operation areas should not have any unwanted raw materials, packaging materials, and others accumulated.

Cleaning and sanitizing tools and supplies should be placed in specially designed areas.

No harmful materials should be placed or stored in the operation site.

Water-holding tanks should be washed periodically to meet potable water standards.

C. Sanitary Management of Equipment

Equipment used for manufacturing, packaging, storage, and transportation should be washed and sanitized periodically. The wash and sanitizing operation must not contaminate the food, the food contact surface, or packaging materials.

Food-contact surfaces should be washed after sanitizing.

After the operations, any used equipment and utensils should be cleaned thoroughly and kept from contamination and in ready-to-use condition. The water must be of potable standard.

D. Sanitary Management of Personnel

Workers in the manufacturing plant should keep their hands clean, and wash with detergent before operation.

Workers who contact food directly should have their fingernails clipped properly and avoid fingernail polish or jewelry.

Hands in direct contact with food to be consumed without further heating should wear previously cleaned and sanitized, water-resistant gloves—or the hand should be cleaned and sanitized.

Workers should wear clean clothes (uniforms preferred) and hats, preventing hair, dandruff, and foreign matters from getting in to the food, on the food contact surface, or inside the packaging materials.

Workers responsible for the soya koji and packaging (before sealing) should wear mouth masks.

During operation, workers must not eat, drink, or smoke.

Sweat, saliva, cosmetics, or medication must not contaminate the food, the food-contact surface, or the interior of the packaging materials.

Workers with communicable diseases or wounds that may contaminate the food should not engage in activities that have direct contact with food.

New workers must pass their physical and health examinations before starting to work. They should be reexamined each year thereafter. Items to be examined should meet the requirements for workers in the areas of food manufacturing, formulation, processing, selling, and storage of food of food additives, and food establishments.

Personal items should be stored in lockers in appropriate locations and should not be brought into the food preparation or equipment- and utensil-washing areas.

Workers must wash and sanitize their hands before going to work or after visiting the toilet.

Entry by visitors to the plant should be properly managed. When visitors enter the restricted operation area, they should meet the sanitary requirements for workers in those areas.

E. Sanitary Management of Manufacturing Process

Establish the standard operation procedures, control procedures (including process flow-chart(s), targets for management control, items to be controlled, control standards and items to be aware of), and standard procedures in operating equipment and maintenance. Follow established procedures during operation.

F. Sanitary Management of Raw Material Treatments

All raw materials and ingredients should be treated to remove the attached micro-organisms and toxic or harmful components, up to the acceptable standard.

When intermediates or products from outside suppliers are used as raw materials, they must meet the sanitary standards for raw materials, manufacturing environment, manufacturing processes, and quality control.

Before the raw materials are used, they should be visibly inspected and graded to remove the defective and foreign matters.

Water used for washing of raw materials must meet the potable standard. Recycled water must be sanitized and filtered to avoid the raw materials being contaminated for a second time.

During storage of raw materials, avoid contamination, damage, and quality deterioration.

Frozen raw materials should be kept at $-18\,^{\circ}C$ or below; refrigeration should be below $7\,^{\circ}C$ and above the freezing point. Use of frozen raw materials should follow the first-in, first-out principle. Thawing of frozen raw materials should be conducted in a sanitary manner.

Removal of sheath and dust from wheat should be conducted in dust-collecting facilities to avoid contamination of the environment.

G. Sanitary Management of Manufacturing Operations

All food manufacturing operations (including packaging and storage) must follow safety and sanitary principles, and be conducted in a controlled environment to reduce microbial growth and contamination within a minimal time.

Food manufacturing operations should control physical requirements (such as time, temperature, water activity, pH, pressure, and flow rate) and processing procedures (such as freezing, refrigeration, dehydration, heat treatment, and oxidation). These operations should ensure the food will not deteriorate or be contaminated due to mechanical failure, time delay, change in temperature, and other factors.

Foods that are vulnerable to multiplication of harmful microorganisms should be stored in a condition that can inhibit the quality deterioration. Refrigerated foods should have a central temperature of $7\,^{\circ}C$ or lower and be held above the freezing point.

Acid food or acidified food in sealed containers designed for room temperature storage should be heated to inactivate the mesophiles.

Use of heat sterilization, irradiation, low-temperature sanitization, freezing, refrigeration, and pH or water activity control to eliminate or inhibit harmful microorganisms can inhibit the quality deterioration of food during manufacturing, storage, and transportation.

In using equipment, containers, and utensils for transfer, transport, or storage of raw materials, intermediates, or final products, the operation and maintenance procedures should not cause contamination in the food. After coming in contact with the raw materials or contaminated matters, equipment, containers, or utensils should be washed and sanitized before use in treating food products. Containers used to hold intermediates during food processing should not be put on the floor: This is to avoid indirect contamination through the bottom of the container or due to splashing of water.

During soya koji making, fermentation, maturing, and cooking steps, it is important to avoid the multiplication of undesirable foreign microorganisms.

A double-checking procedure should be established for weighing and addition of food additives.

During manufacturing, the intermediates and the products depend on pH level, salt content, and sterilization to maintain safe conditions. These factors should be under stringent control to avoid quality deterioration.

Effective procedures are required to protect the food in processing or storage from contamination due to raw materials, wastes, metals, or other foreign matters.

Stringent control on blanching temperature and time are required for blanching, with rapid cooling. At the same time, periodic washing of blanching equipment is needed to avoid

growth of heat-resistant microorganisms and their contamination. Food that requires cooling before filling must be cooled with potable water.

Acid food or acidified food depends on pH to control growth of harmful microorganisms in the food. Adjustment of the pH to 4.6 or below can be achieved by adjusting the pH of raw materials, intermediates, and product, or by controlling the quantities of acid food or acidified food added to the low-acid foods.

The inner packaging materials should be selected based on their ability to effectively protect the food during storage, transportation, and marketing from the entry of harmful materials and to meet the sanitary requirements.

In general, food containers are not recycled except for glass or stainless steel containers. However, they have to be washed and sanitized before use.

Defatted soybeans require proper high heat treatment. Wheat has to be roasted and broken (30% passing through a No. 30 sieve, with control on expansion rate and breakage rate). During the soya koji–making process, temperature should be controlled between 25 and 40°C, with periodic turnover to facilitate mycelium growth. The brine for preparing the soy sauce mash should be below 15°C. Its concentration has to be prechecked and suitably adjusted. The fermentation of the soy sauce mash should be conducted in a closed system, with regular control of temperature, mixing (according to mixing plan), fermentation, and maturation. Sanitary control of the filtering system and the cloth used is also important, avoiding contamination with foreign microorganisms and foreign matters. Cooking (sterilizing) temperature and time have to be controlled. Equipment has to be washed and sterilized after use. Sterilized soy sauce after sedimentation, can be packaged into final products. Introduction of foreign material needs to be avoided.

V. QUALITY CONTROL: ESTABLISHMENT OF THE QUALITY CONTROL MANUAL (87)

A. Quality Control of the Raw Materials

Establish a system to evaluate and track the supplier for raw materials. Establish the quality standards, items for inspection, inspection standards, sampling plan, and inspection procedures. It is critical that the control system be executed. Every lot of incoming raw material has to be inspected and passed before being accepted for use in the plant. Every lot has to be sampled according to the plan. The inspection can be regular, reduced or enforced to assure composition, purity, and content match the written specifications. Suppliers for packaging materials should provide regular safety and sanitary reports. If the raw materials contain agricultural chemicals, heavy metals, or aflatoxins, it is critical that their concentrations should be within limits before they can be used. Use of accepted raw materials should follow the first-in, first-out principle. Rejected raw materials should be properly labeled as "Forbidden To Use" and stored separately. The use of raw animal materials to manufacture amino acid liquid (protein hydrolysates) is absolutely forbidden.

B. Quality Control of Food Additives

Food additives should be stored in special cabinets. Expiration dates should be observed. Food additives should meet sanitary standards. Their use should follow the rules and regulations governing the use of food additives in different countries. Incoming food additives should have proper labels with permission forms checked accordingly. Food additives with controlled usage (such as preservatives and antioxidants) should be stored separately. Their usage should meet legal requirements (86).

C. Quality Control During Processing

Identify the critical control points for safety and sanitation and establish their inspection items, standards, sampling plan, and examination procedures. This plan must be executed accordingly. Quality control during processing should include raw materials, intermediates, and final intermediate products. Raw materials such as soybean require protein denaturation test, total nitrogen, and moisture content. Examples of intermediates are raw soy sauce and amino acid liquid (protein hydrolysates). For the final intermediate product, inspection includes soluble solid content (in ^{0}Baume or ^{0}Brix), pH value, salt content (%), formaldehyde nitrogen (FN %), total nitrogen (TN %), and color. Check to see if these results meet the required standards. For mixed soy sauce, analysis of the main components is required to adjust the ratios accordingly in order to guarantee that the main components will meet the requirements.

D. Quality Control of the Final Product

Establish the quality standards, inspection items, limits, sampling plan, and examination procedures. It is important to save samples from every lot for future reference. If necessary, storage tests may be needed to determine storage stability. Products not meeting the standard should be treated accordingly. The final product must not contain toxic or harmful substances, or foreign matter. It should also meet the legal sanitary standard. Raw materials, intermediates, and final intermediates should be labeled and treated accordingly.

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Fermented Whole Soybeans and Soybean Paste

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I. INTRODUCTION

Sorted by product shape, itohiki-natto, hamanatto, and douchi are fermented whole soybean products; dou-pan-chiang comprises particulated fermented soy foods; and miso is closer to a thick pastelike product. However, if classified according to used microorganism, Itohiki-natto uses bacteria, whereas hamanatto, douchi, dou-pan-chiang, and miso utilize molds in their manufacture.

When we use molds in fermentation, we generally prepare the seed starter and then inoculate the starter to raw materials for fermentation. The raw material for this seed starter is the substrate for the mold. The typical example is the seed koji used in the manufacture of fermented foods. Seed koji is made by culturing the mold spores on cereals such as rice or wheat, and then drying them for long-term storage.

In the fermentation industry, we use pure cultures. However, in the fermentation process, most manufacturers use an open management system. Therefore, other microorganisms from the environment will grow and participate in the fermentation process when conditions are suitable, thus affecting product quality. In the manufacturing of fermented soy foods, large amounts of salt are applied. This situation provides a selection criterion by eliminating microorganisms that are not salt-tolerant and allows fermentation to go on smoothly. Among the different salt-tolerant microorganisms are many salt-tolerant yeasts and lactobacteria. In general, these salt-tolerant microorganisms have positive effects on the product quality of these fermented foods. Therefore, many types of fermented foods are made from the combined actions of molds, salt-tolerant bacteria, and yeasts, with production of characteristic flavors.

II. NATTO (ITOHIKI OR ORDINARY NATTO)

A. Introduction

Natto is an ancient Japanese fermented soy food. It is consumed directly without further heat treatment. It is made by inoculation of *Bacillus natto* onto cooked soybean. After fermentation, the final product is characterized by its slimy covering appearance. There are

about 1000 natto manufacturers in Japan with annual production of 50,000 metric tons. Most manufacturers produce about 150 kg per day; a few produce over metric 1 ton.

B. Manufacturing of Natto

1. Raw Materials

The main ingredients for natto manufacturing are soybeans and natto inoculum.

a. Soybeans In general, soybeans for natto manufacturing should be small (6.6 mm diam.) to medium (6.6–7.2 mm diam.) size, with softer texture and more fermentable sugars. The cultivars more suitable for natto manufacturing are Jing Shan Pu, Hulan, Feng Shou from China, and Ziyusiyo Long Leaf from Japan. From the size standpoint, the smaller the beans, the better product. This is because the smaller beans absorb more water and are easier to cook. The more water is absorbed, the better the production rate. Besides, the fermentation is aerobic. The smaller beans provide a better aerobic environment. Because the properties of soybeans from different cultivars vary considerably, it is recommended not to mix different cultivars, even if they are of the same size (Fig. 1).

b. Natto Inoculum The natto inoculum is *Bacillus natto*, formerly classified as *Bacillus subtilis*. However, *B. natto* requires biotin to grow and develop properly, whereas *B. subtilis* does not have this requirement, even though the other characteristics are very similar. *B. natto* is a gram-positive bacteria found commonly on rice straw and cereal grains. Its spores are very heat-resistant. This bacterium can be isolated by the following procedure. The above materials are boiled in water for 10–20 min. This will kill most of the other microorganisms. *B. natto* can then be isolated from the boiled liquid by regular pure culture–isolating techniques. Cells from the pure culture can be washed and suspended in inorganic liquid or dried to a powder for commercial retailing of natto seed inoculum.

2. Treatment of Raw Materials

A flowchart on Itohiki natto manufacturing is presented in Fig. 2.

a. Selection and Washing Raw soybeans are selected through a sorting machine to remove foreign matters, broken beans, stones, and dust to select similar-sized soybeans. They are then washed to remove the dirt and dust adhered to the surface.

b. Soaking In order to assure the beans will be cooked and softened easily, it is necessary for the beans to absorb enough water. The amount of water absorbed should be

Figure 1 Marketing natto.

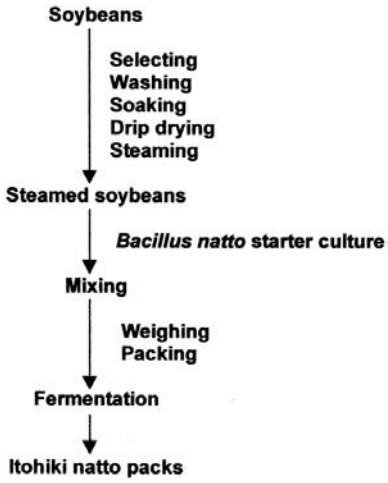

Figure 2 Flowchart on Itohiki natto manufacturing.

2.2 times the weight of the dry beans. The cotyledons, after water absorption, should be tightly attached to each other when the bean is cut horizontally. The soaking time varies with the age of the beans, water temperature, cultivar, and size. Soaking time may be 27 to 30 hr at 2.5°C in the winter, 18–21 hr at 13°C in the spring, and 10–14 hr at 21.5°C in the summer (1). The soaking water should be of potable quality. Water with higher hardness and iron (ion) content above 5 ppm should be treated to soften the water and remove the iron ions. Cooking of beans in hard water makes the cooked beans tough, and iron ions will blacken the beans.

c. Cooking Soaked beans are drip-dried before steaming. Nowadays, most producers use pressurized steam cooking. A pressure cooker handling 100 kgs raw beans at one time is more common. Formerly, the cooking was a still process (Fig. 3). Nowadays, a rotating cooker is used. Beans are loaded in the cooker in batches with steam on. When the

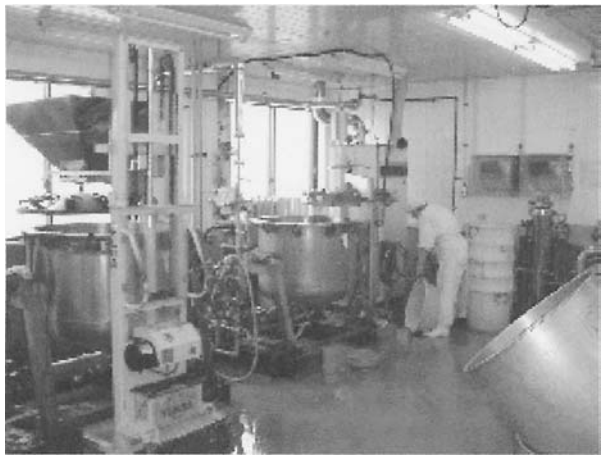

Figure 3 Raw materials cooking equipment.

steam penetrates evenly into the center of the beans and starts to come out, the venting valve is closed, and a pressure of 1–1.5 kg/cm^2 (121–127°C) for 20–30 min is maintained. Then the venting valve is opened slightly to the start cooling down of the beans. With this procedure, part of the amino acids or sugars will be hydrolyzed, causing loss of nutrients. The color of cooked beans will also be darker, affecting the product quality. In practice, a pressure of 0.8–1.0 kg/cm^2 for 30–40 min is a better alternative. The latter procedure will let the natto bacteria grow easily on the cooked bean.

3. Inoculation with Natto Bacteria

The cooked beans are taken out from the cooker. The natto starter is inoculated immediately and mixed thoroughly. The amount of inoculum is 1 kg of raw beans to 1 ml liquid inoculum or 1 g inoculum powder. When liquid inoculum is used, the starter is diluted with 100 ml of sterilized water before inoculation. Commercial natto starter contains bacteria in the spore form. One ml or 1 g of this starter has 10^8 spores or more. When it is used to inoculate small soybeans (6500 beans per kg), each bean will have at least 10^4 spores. This is extensive inoculation (Fig. 4).

Temperature of inoculation is usually 80°C for the beans. This can avoid contamination. If the facility is very sanitary, inoculation at 45°C is adequate. The inoculation is usually conducted in a mechanical inoculator. The cooked beans and the liquid inoculum are put into the inoculator, followed by rotation to ensure even mixing. Or the inoculum can be added to the beans, followed by stirring.

4. Packaging and Weighing

Inoculated beans are put into the proper container for fermentation. This process is characteristic of natto manufacturing. Because the inoculated beans have to be put in one by one, the packaging and weighing are very labor intensive. The process has to be mechanized (Fig. 5).

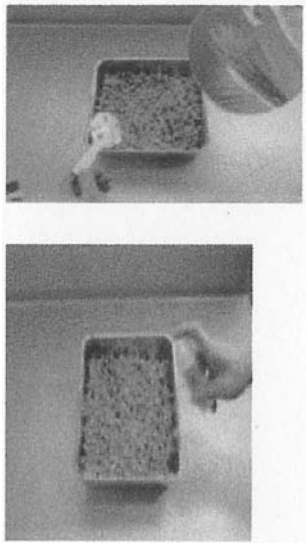

Figure 4 Inoculation of *Bacillus natto*.

Figure 5 Packaging and weighing.

In the past, the package material was rice straw. Later, thin wood slices of fir or cypress was used. Nowadays, bags with perforated inner lining of polyethylene film, or polystyrol containers are used. Another type of container is made from rice straw mat. Failure in natto manufacture is mainly due to contamination with foreign microorganisms. Inadequate sterilization of the container is one of the major causes. Therefore, proper sterilization of the containers is critical. Containers made from wood slices, rice straw, or mats should be steam-sterilized. Synthetic materials such as polystyrol cannot be heat sterilized and therefore are sterilized by fumigation with sulfur, ethylene oxide, or other government-approved sterilants. The amount of natto per container is usually 0.1 kg.

5. Incubation Room

A standard incubation room for natto has a door with an internal dimension of length 2.4 m × width 3.3 m × height 2.1 m. The roof has a shape of inverted bottom of a boat, with a vent in the center. The door should be a double-door design. The wall should be surrounded with wood panels or stone. Mortar is put on the surface or it is lined with iron sheets. If insulation is needed, a 25-cm thick layer of sawdust, fiberglass, or polystyrol can be used. Inside the room, shelves are installed on both sides, with steam pipes or electrical heaters near the beds to provide a heat source. This kind of incubation room can hold 3500 units of 0.1 kg packages. Recently designed equipped with controls to automatically adjust the temperature and relative humidity.

6. Fermentation and Its Management

The optimum germination temperature for *B. natto* spores is about 40°C. The spores can still germinate after storage at 0°C for one year. In actual practice, the cooked soybeans are inoculated at about 80°C. Fortunately, the *B. natto* spores are heat-tolerant and can survive this exposure to high temperature. This heat-shock helps induce the dominant spores to germinate. After exposure to 100°C for 3 mins, or 80°C for 30 mins, the spores start to germinate. It is important that the beans be immediately cooled to about 40–50°C for proper germination and multiplication. Overexposure to high temperature such as 100°C for 30 min, at this stage will eliminate the germination capability of the spores. The germinate spores also are not heat-tolerant. The bacteria will not grow at temperatures higher than 55°C. Growth at 20°C is only one-tenth that at 40°C.

Growth of *B. natto* is also affected by pH of the substrate. It grows best in a neutral or slightly alkaline environment. Cooked soybean has a pH of 6.4 to 6.8 and increases to

7.2 to 7.6 as fermentation progresses. A pH value of 4.5 has significant inhibitory effect in growth of *B. natto*.

Oxygen also affects the growth of *B. natto*. In actual practice, carbon dioxide concentration in the fermentation/incubation room can be over 15%. However, it has little effect on the maturation of the natto soybeans.

The *B. natto* bacterium grows easily on cooked soybeans. It can also grow on other animal or plant foods. However, it grows better on plant protein foods and produces more sticky substance.

Carbon sources such as glucose, fructose, and sucrose can be used easily by *B. natto* for growth. Sucrose is also needed for the production of sticky substance. Soybean contains about 20% carbohydrates, of which 30% is sucrose. This is plenty for the growth of *B. natto*.

The nitrogen source for growth of *B. natto* is proteins and amino acids. *B. natto* can easily utilize glutamic acid, arginine, aspartic acid, and proline but not threonine, tryptophan, phenylalanine, and methionine. In soybeans, there are more amino acids that *B. natto* can utilize than ones it cannot.

Bacillus sp. such as *B. subtilis*, *B. megaterium*, *B. cereus* have no requirement for biotin for growth; however, other *Bacillus* sp., such as *B. natto*, *B. mycoides*, *B. pumilus*, and *B. coagulans*, do. *B. natto* does not germinate without biotin. It requires at least 0.1% (0.001 ppm) for minimal growth and 1% (0.01 ppm) for proper growth. Soybeans have 60 g biotin per 100 g. Other B vitamins are abundant in soybeans and they are also beneficial to *B. natto* growth (2).

Fermentation of natto is greatly affected by the temperature of soybeans entering the incubator and the temperature and relative humidity of the incubator thereafter. When the initial temperature soybean is cooled below 40°C, germination of *B. natto* will be delayed and the time to increase bean temperature will be extended. This is the critical time when contamination can occur most easily. It should be shortened by all means to ensure safe fermentation. Therefore, the actual practice is to have the beans at about 42°C when entering the incubator with 85–90% relative humidity. After 2 hr, the *B. natto* spores will germinate and produce heat. At 6–8 hr, maximum heat is generated from fermentation.

Temperature of the fermenting beans will reach about 48°C. Thin, white film of the bacteria is visible. The characteristic odor of natto and sticky substance start to develop. When the temperature of fermenting beans reaches 50°C, supplemental heating should be discontinued. Relative humidity too low will produce hard natto beans. Therefore, relative humidity in the incubator should be maintained at 80–85% and temperature at 50°C. The maturation period is about 16–18 hr. At the end of this period, the natto beans should be taken out from the incubator to cool down. They should be delivered for marketing the same day. If it is necessary to delay the delivery, holding the product at 5–10°C is necessary.

C. Changes During Natto Fermentation

1. Changes in General Composition

During an 18-hr natto fermentation, the reducing sugars decrease by about 15% after the sixth hour. There is essentially no change in crude fat, crude fiber, and ash contents (1). However, the protein is digested gradually, even though the total nitrogen stays essentially the same.

2. Changes in Characteristic Flavoring Compounds

The characteristic flavoring compounds of natto beans are produced during fermentation. The umami compounds are mainly the protein hydrolysates. During natto fermentation, about 50–60% of the proteins soybean are hydrolyzed to water-soluble nitrogenous compounds, of which 10% is amino acids. Water-soluble nitrogen increases by 228%, amino nitrogen by 757%, and ammonia nitrogen by 900% (dry wt. basis) (1). The free amino acids profile also changes considerably. The freeing rate ranges from 2% to 26%, with leucine, alanine, tryptophan, and threonine being the highest, and aspartic acid, proline, serine, tyrosine, and cystine being the lowest. Glutamic acid is related to the umami taste, and it has a freeing rate of 10%, with 0.36 g per 100 g natto beans (1).

Selected component changes in natto beans are shown in Table 1.

Organic acids are also related to the flavor of natto beans. Acetic and lactic acids have the highest concentration among all the organic acids in natto beans, but show little changes during the fermentation process. Butyric, propionic, and succinic acids increase during fermentation to 0.078%, 0.007%, and 0.009%, respectively (2). Natto beans made in rice straw containers have more butyric acid than the other packages. The off-odor or bitterness in stored natto beans is the result of mold growth on the surface of natto beans with the prodution of peptides with isoleucine terminal, tyrosine crystals, and the m/ odorous isolvaleric acid. The off-odor of stored natto beans is also related to the ammonia, organic acids, fatty acids, and other diacetyl compounds. Diacetyl increases during natto maturation but decreases during storage. When tetramethylpyrazine in natto beans reaches a certain level it will also induce off-odor.

3. Sticky Substance

The mechanism that forms sticky substances in natto beans is still not completely understood. However, it is known that the substance is made up of glutamic acid polypeptide and fructose polymers. The polypeptide has a M.W. of about 15,000 with about 100 units of glutamic acid. Ratio of the polypeptide and the polymer in the sticky substance varies according to samples. The polypeptide usually is about 60–80% of the sticky substances, with strong thread-forming capability, and the fructan stabilizes the stickiness. The amount of this sticky substance is equivalent to 2% of the dry weight of natto beans. It is most stable at pH 7.2–7.4. The stickiness of this substance is thinner at pH values higher or lower than this pH range. For example, addition of salt during food preparation will

Table 1 Changes in Selected Components of Raw Soybean, Cooked Soybean, and Natto Bean

Component	Raw soybean	Cooked soybean	Natto bean
Total nitrogen	100	100	100
Water-soluble nitrogen	31	11.2	58
Water-soluble nitrogen in 10% brine	16	1.7	8
Soluble nitrogen in 75% alcohol	0.8	0.9	2.5
Soluble nitrogen in 0.2% NaOH	11	22	17.5
Insoluble nitrogen	41.2	64.2	14

Units in percent.
Source: Ref. 2.

result in a stickier product as compared to use of soy sauce. The stickier natto is more vulnerable to foreign microbial contamination or *B. natto* phage contamination.

4. Enzymes in Natto Beans

Natto beans have a microbial population of 10^8/g. The natto bacteria produce different enzymes. Among them, proteases are the majority. Alkaline protease reaches an amount that can be crystallized. At pH 8.2 and at 55°C, it has the highest activity. It is also stable between pH 5 and 9. It is not stable at temperature above 55°C. Heating at 65°C for 30 min can inactivate this enzyme. The activity of this enzyme is stronger than the hydrolytic powder of commercial pancreatin on casein. Other enzymes such as amylase, lipase, and cellulase are also very active.

5. Nutritive and Pharmaceutical Values of Natto Beans

Natto beans are very nutritious. Natto is a fermented soy product and a protein source. Because the soy proteins are hydrolyzed, natto beans can be digested more easily. Given feed made by mixing polished rice flour (68%), natto beans (28%), and inorganic salts (4%), white mice were found to have an absorption rate of 93.2%. In natto beans, 86.8% of the protein, 89.9% of the fat, and 97.4% of the sugar can be absorbed. Natto is therefore better than cooked soybeans.

Vitamin B_2 content in natto beans is 5–10 times more than that of the unfermented beans, with about 1 mg in 100 g of natto beans. Vitamin B_1 loses its activity during the cooking of the beans, but it gains back 30% of its activity after fermentation. The digestive enzymes in natto beans can help prevent epidemic diseases that affect the digestive tract, such as dysentery and typhus. It is also helpful in the prevention or dietary treatment of food poisoning and intestinal diseases.

D. Product Quality

Good natto beans should have uniform size. They should be covered with grayish white film of *B. natto* and be brightly colored. They should be free of discolored spots. The beans should stick together, showinga soft block with stickiness. The sticky substance can be pulled into long threads. The beans should have a characteristic odor with no off-odor (3).

III. HAMANATTO

A. Introduction

Japanese hamanatto is a fermented, intermediate-moisture soybean that belongs to a group of very similar, fermented soybean products that are sometimes called soy nuggets. It is made by fermenting the cooked soybeans with *Aspergillus oryzae* followed by soaking in a brine or soy sauce, and then dried. It is consumed directly in Japan. It differs from the ordinary natto with its sticky substance on its surface. In Japan, various similar names are used with this type of products such as daitokuzi natto, diyohukuzi natto, and hamanatto, depending on where they are produced. The product is salty and has a black color. A very similar Chinese product is douchi (see below) that is used as seasoning in food preparation or processed with other seasonings such as garlic, and packaged in jars. In this section, the manufacture of hamanatto is discussed.

B. Manufacture of Hamanatto (2)

A flowchart on Hamanatto manufacture is presented in Fig. 6.

1. Raw Materials

In the manufacture of hamanatto, the basic raw materials are soybeans, wheat, salt, water, and the inoculum *Aspergillus oryzae*. The soybeans from Hokkaido (Japan) are used in Japanese production. Wheat and sometimes barley are used. Salt is the commercial crude salt. Ginger is added in the seasoning of soy sauce used for soaking.

2. Raw Material Treatments

Uniform soybeans are selected by removing the foreign matter, imperfect beans, stones, and dirt. The beans are washed to remove the adhering dirt and dust, and soaked for 3–4 hr at 20°C and the drip-dried. The beans are steamed at ambient pressure for 5–6 hr and let cool in the cooker overnight. Or a rotary cooker can be used to steam cook the beans at 0.8–1.0 kg/cm^2 for 30–40 min. The selected wheat or barley is roasted (or stir-fried),

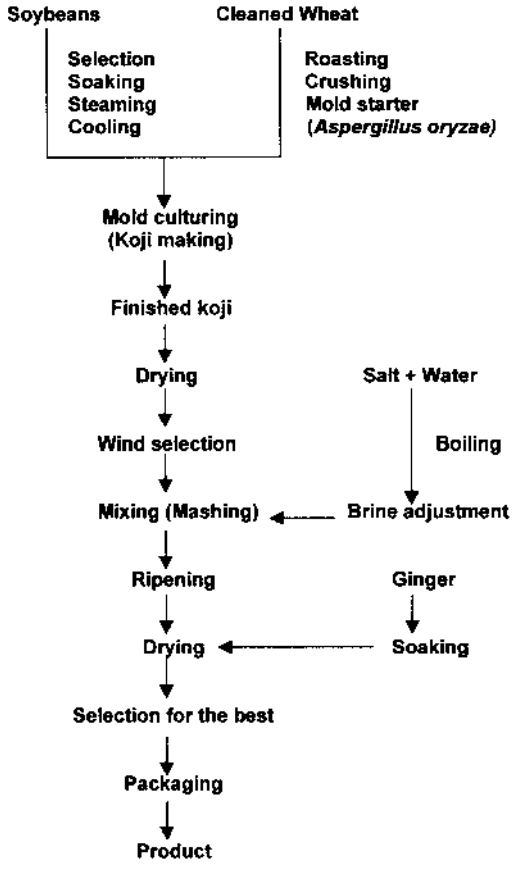

Figure 6 Flowchart on making Hamanatto.

followed by crushing and sieving to pas through a No. 85 sieve, or a No. 30 mesh sieve with at least 30% powder pass through.

3. Ratio of Raw Materials

The ratio of soybean: wheat powder: salt: ginger should be 100: 9.2: 18: 7.5 for the manufacture of hamanatto.

4. The Manufacturing Process

The cooked soybeans are transferred to the koji room and spread thinly to cool down to below 40°C.

The wheat and barley (barley flakes: wheat bran at 1:1 ratio) or brown rice are steamed and mixed with potash. The seed koji starter is prepared by mixing 0.1% of the *Aspergillus oryzae* spores with the roasted (or stir-fried) wheat powder. The seed koji starter–wheat powder is then mixed with the cooked wheat, barley, or rice. The mixture is incubated for 3–4 days to form the koji starter. This koji starter is mixed thoroughly with the cooled soybeans and left on trays for fermentation at 30–33°C for 50 hr.

The fermented beans are sun-dried to reduce the moisture content to about 22%. These dried beans are soaked in 15° Baume (17.4% sodium chloride) brine for 8 months. A weighing stone may be needed to hold the beans below the brine. Another alternative is to use soy sauce as the soaking medium instead of brine. When they are ready, the beans are taken out for sun-drying on a hemp cloth bag. In addition, ginger soaked in sauce is added at a ratio of 7% to form the final product.

C. Product Quality

Hamanatto is a dull brown, without luster, but has a strong umami odor. It has the following general composition: 36–38% moisture, 26% protein, 12% fat, 6–7% reducing sugar, 12% ash (including 11% salt), and 2.1–2.9% crude fiber. Also, it has about 0.86% formaldehyde (amino group) nitrogen, 2.5–2.6 water-soluble nitrogen, 31.22–39.22 acidity I (related to umami taste of amino acids), 24.70–31.72 acidity II (related to buffering capacity of phosphoric acid), 34–35% extract (soluble solid components), 30–32% water-insolubles, a pH of 5.10–5.15, and 255 Kcal (8).

IV. DOUCHI

A. Introduction

Douchi is a Chinese fermented soybean product. It is very similar to the Japanese hamanatto. Douchi can be made from black soybean (preferred), or soybean. One kind of douchi, sometimes called yinshi, is the dried residual black soybean koji (starter) in the manufacturing of Yinyui (pot bottom oil or pot bottom excrement, one kind of soy sauce made from black soybean, *Glycine max* L. merri var. o au). Good-quality douchi is made by proper control of the bean soaking and steaming processes so that the beans absorb the right amount of moisture. Usually, the moisture absorbed is less than the amount used to make soy sauce; therefore, it is harder after steaming. The manufacturing process is the same as in the manufacturing of soy sauce using black soybean.

Another kind of douchi is manufactured using a different procedure. The black soybeans are inoculated and form the koji. The koji is then washed, dripped dried, and

incubated to bean temperature of 38–40°C. The beans are stacked between layers of sweetened wine with a top layer of salt. The beans are then sealed in the containers for ripening. The fermentation usually takes about a month. If the final product is too wet, they can be sun-dried to adjust the moisture content.

Douchi is a very suitable seasoning for cooking seafoods. It can eliminate the fishy flavor and at the same time provide a sweet taste. It is also used in the seasoning of meat dishes. Seasoned Douchi products with garlic, ginger, chili pepper, and/or stir-fryed in flavored oil are also now available for food preparation.

B. Manufacturing of yinshi-type Douchi or Doushi

A flowchart on douchi (yinshi) manufacturing is presented in Fig. 7 (4,6).

1. Raw Materials

a. Black Soybean (Glycine max L., Merri var. o'Tau) Raw black soybean used for making douchi should be well-developed, properly dried, and free of insect damage, dirt, and other foreign material. Composition of black soybeans from different sources varies slightly (Table 2).

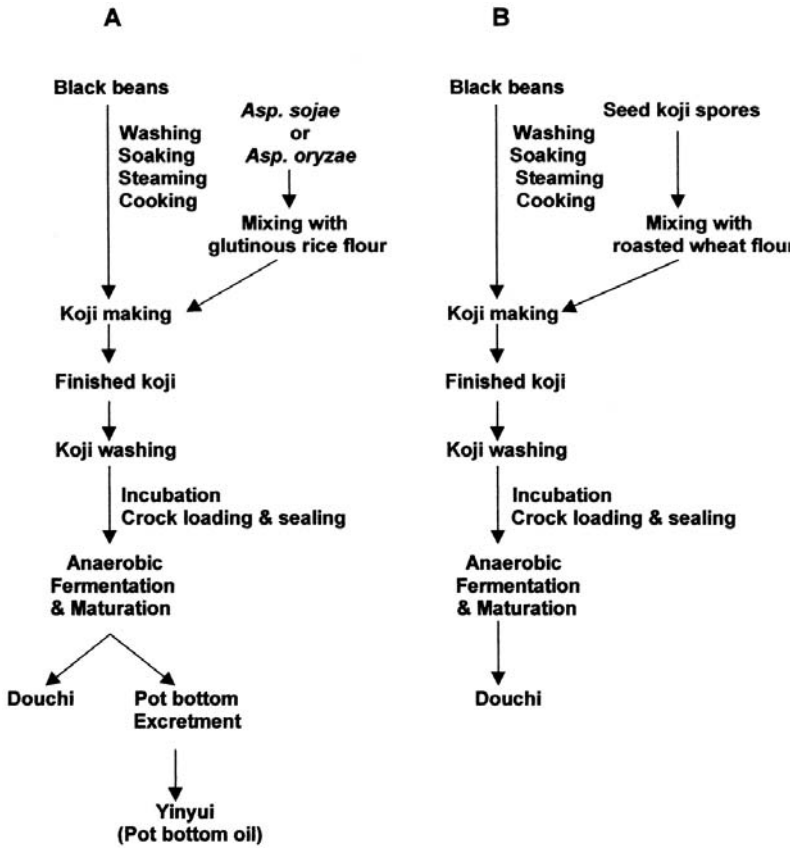

Figure 7 Flowchart on making Douchi.

Table 2 Composition of Black Soybean from Taiwan (R.O.C.) and Thailand

	Composition (%)						
Source	Moisture	Crude Protein	Crude Fat	Carbohydrates	Crude Fiber	Ash	Foreign Matters
Taiwan	12–13	38–42	13–21	11–27	3–3.5	4–5	2.0–3.5
Thailand	12–13	35–37	13–21	12–29	3–3.5	4–5	2.0–2.5

Source: Ref. 5.

b. Salt Either crude or refine salt is suitable for manufacturing douchi (Yinshi). The amount is about 20% of that for black soybeans.

c. Glutinous Rice In the manufacturing of yinyui, about 1–2% glutinous rice or Taiwanese rice is used. The purpose is to provide some stickiness in the final product. In the manufacturing of douchi only, about 0.3% of roasted (or pan-fried) wheat flour is used in the preparation of the starter.

d. Seed Starter (Koji) The seed starter is either *Aspergillus oryzae* or *Aspergillus sojae*. *Asp. oryzae* has higher amylase, acid protease, and acidic carboxyl peptidase activity, whereas *Asp. sojae* has higher endo-poly-galacturonase and glutaminase activity. The amount of spores used is 0.5% (w/w) that of black soybeans.

2. Raw Material Treatment

a. Washing and Soaking of Black Soybeans Selected and weighed black soybeans are put in a perforated container and washed with water to remove the soil and dirt adhered to them. The beans are soaked in water for 3–4 hr (2–3 hr in the summer, and 4–5 hr in the winter) until the water absorbed by the beans is 1.8–2.0 times that of the weight of the beans (i.e., the soaked beans weigh 180–200 kg for 100 kg dried beans). Ferrous sulfate, used in the past to make the final product blacker and brighter with better appearance, is not recommended nowadays because of its toxicity.

b. Steaming of Black Soybeans After soaking, black soybeans are adequately drip-dried. Excessive moisture will create problems in the starter preparation stage. Soaked beans can be cooked with pressurized steaming, ambient pressure steaming, or closed-pot boiling and holding. For pressurized steaming, the drip-dried, beans are loaded into NK chambers for steaming at 113°C for 30 min or at 117°C for 20 min. For defatted black soybean flakes, the steaming process is at 131°C for 5 minutes. Steaming of black soybeans is easier than of regular soybeans, and they cannot be overcooked. Extensive absorption of moisture will create problems at the starter preparation stage, producing undesirable odor and sticky compounds.

For ambient pressure steaming, soaked beans on trays are loaded or to the steamer for 2–3 layers. After steam appears from the top layer of beans, continue to steam for another 2 hr. The beans do not have to be cooked to too tender but have to be completely cooked, without the raw flavor when chewed.

For the boiling and holding method, soaked beans are put into the pot. The water added is about 90–100% of the volume of the beans. It is heated until fully boiling and then the lid is put on. Cooking of the beans is continued with medium heat for 70–90 min. The cooked beans are taken out and cooled on trays. The beans have to be turned over frequently to help evaporate the water to the point that they are somewhat dry and not sticky. These beans are then ready for making the starter.

3. Preparation of the Starter

a. Mixing the Inoculum with the Beans The wheat flour is roasted (or pan-fried) to yellowish brown and let cool. Extensive holding of the roasted (or pan-fried) flour is not recommended as this flour can absorb moisture and be easily contaminated. Usually, 1–2 kg of flour is used for 100 kg black soybeans. The roasted (or pan-fried) flour is thoroughly mixed with the inoculum spore before mixing with the cooled beans. When the cooled bean is too soft or contains too much moisture, 3–4 kg of flour will be needed to adjust the moisture content of the mixture. When the beans are mixed with the inoculum, each bean has to have inoculum spores on its surface. Beans without the inoculum spores are easily spoiled due to contaminated bacteria. If wheat flour is not used, the amount of inoculum spores should be increased to 150 g for 100 kg of beans (0.15%) (Fig. 8).

b. Preparation of the Starter Koji In general, the procedures for making soy sauce can be followed (see Chapter 29). However, the incubation time will be extended to 5 days. This is longer than required for soy sauce making. For ancient methods, the duration is 7 days as seed starter is not used. The mixture is left in the sun for cooling (without temperature maintenance) and drying for a suitable period. This will adjust the moisture content and also allows for growth of other desirable microorganisms from the environment. At the end of this step, growth of *Rhizopus* and *Mucor* is usually visible.

4. Washing of the Starter Koji

At the end of the previous step, lots of spores are formed already. In the ancient procedure, lots of other microorganisms are present and create a bitter taste. A washing step to remove these spores is therefore needed. This step is not needed in the making of soy sauce using regular soybeans. However, when black soybeans are used for making yinyui-type soy sauce, the black soybeans are more tender and wheat flour is not used to adjust the moisture content of the raw material. Spores are inoculated directly onto the surface of the beans. The mycelia can enter the beans easily. Space between the two cotyledons will be full of mycelium and spores. Therefore, the spores on the surface of the beans will significantly affect the final product quality. If washing is not conducted before

Figure 8 Making of Douchi koji.

loading into the crock for maturing, the mold odor and bitter taste will be too strong, affecting the flavor of the final product.

The current practice is to use a semi-mechanical washer. The washer is filled with 20–30 kg of water followed by 10–15 kg of the starter koji. The washer is manually rotated for 1–1.5 min to mix the water and the koji (Fig. 9). The mixture is decanted into perforated baskets and washed again rapidly with 4–5 kg of water by pouring the water through the beans from the top. The washed beans are drip-dried for 2 minutes. This procedure will lose 3–4% of the total nitrogen. The beans will now weigh 50% more as compared to the starter koji, or 1.5 times the original weight of the koji.

5. Incubation

Twenty-five kilogram of washed koji is put into a 30 kg capacity perforated basket. Except during the summer, covering with cloth to maintain temperature is required. This incubation period is 3 hr in the summer and 8 hr in the winter, with holding period adjusted according to temperature in the environment. Heat will be generated from the upper and middle section of the beans. The beans are rinsed with 40–45°C water to allow second absorption of water. They are mixed and held to permit second heat generation. After this step, temperature of the bean koji is adjusted either by digging a hole to vent off the heat in the summer months or taking off the covering cloth in the winter. The temperature of the beans should be maintained below 38–40°C. Temperature too high will lower the activity of proteases in the koji. The complete process of incubation takes 6–8 hr in the summer and 16–24 hr in the winter months.

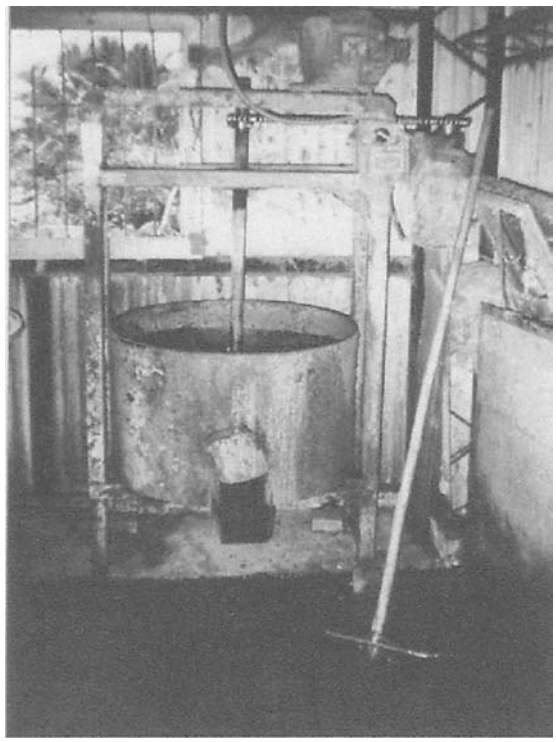

Figure 9 Washing of Douchi koji.

6. Crock Loading and Fermentation

After the incubation step, the koji is mixed thoroughly with 17% salt, and loaded into the crock followed by putting in another 3% salt on the top. The beans are pressed evenly. The crock is covered with the lid and sealed with a mixture of lime and sand. The sealed crock is left in the sun for the bean koji to mature. It takes about 1.5–2.0 months in the summer and 2–3 months in the winter to recover the yinyui or douchi. When the crock is opened, the undissolved salt is collected to avoid the salt getting into the Yinyui and making it too salty. Yinshi not soaked in the yinyui is recovered and transferred to another container. A hole is dug in the center of the yinshi to drip out the yinyui to the bottom. After some time, the yinyui will be dripped to the bottom and the dry yinshi is left at the top.

Another procedure is to layer the washed bean koji in the crock with a small amount of sweetened wine (sugar: rice wine ratio of 1:3). For the two top layers, salt in the amount of 20% of the washed bean koji is sprinkled in. The crock is filled to 80% full. The bean koji is pressed evenly and sealed in the crock. The crock is left in the sun for 1 month to mature. When the recovered product is too wet, it is sun-dried accordingly to adjust the moisture content.

When the bean koji is loaded to the crock, the temperature of the bean koji inside the crock is not that high when left in the sun. However, in the summer months, small amounts of the bean koji at the top and around the crock interior can reach a temperature of 42–43°C. The rest can only reach 35–36°C.

During the washing and incubation treatment, no salt is added. The enzymes are already working actively. When the bean koji is loaded into the crock, only a small amount of salt is added. Therefore, the fermentation (maturation) time is only 1.5 months in the summer, and 2–3 months in the winter. However, the longer the maturation, the better the flavor. When extended maturation is desirable, additional salt at the top of the bean koji is recommended to safeguard the process. Because the lid of the crock is sealed with a sand and lime mixture, the environment inside is not completely anaerobic. When the salt addition is not enough, the top 5 cm layer may be subject to microbial spoilage (Fig. 10).

Figure 10 Fermentation of douchi in crock.

7. Microbial Activities During Fermentation

At the time of loading the crock, the bean koji carries salt-tolerant bacteria, yeasts, and lactic acid bacteria at the level of 1.3×10^5/g, 3.0×10^6/g, and 2.2×10^6/g, respectively. After 14 days of fermentation, salt-tolerant bacteria can increase to 3×10^9/g and then decrease gradually to the 10^6/g range. With the gradual increase of salt-tolerant yeasts and lactic acid bacteria, the salt-tolerant bacteria decrease gradually to 3×10^4/g. The latter is at a lower level than the salt-tolerant yeasts, but still higher than the salt-tolerant lactic acid bacteria. At the end of fermentation, the salt-tolerant yeasts stay at a level of 2.2×10^6/g, and the salt-tolerant lactic bacteria at 6×10^3/g.

During fermentation, the enzymatic activities are as follows: lipase > amylase > protease. At the time of loading the crock, the enzyme activities for lipase, amylase, and protease are 1.4×10^5, 1.3×10^2 and 2.3×10^2 units/g mash, respectively. These enzymatic activities decrease with time of fermentation. This is especially true for protease. It decreases by one-half after 14 days of fermentation and stays at this level until the end of fermentation. During the whole fermentation process, lipase activity is about 1000 times more active than protease and amylase.

8. Compositional Changes During Fermentation

During fermentation, the proteins in black soybean are hydrolyzed by the exoprotease from the mold amino acids. These amino acids are then hydrolyzed by the deaminase and decarboxylase into ammonia and amines (7). With the increase in fermentation time, the total nitrogen in the inyui increases significantly. This trend also applies to formaldehyde nitrogen and amino nitrogen. The hydrolytic rate of proteins can reach 63%, and the total nitrogen utilization rate can reach 69%. Total amino acids increase from 0.23% to 1.8%, with glutamic acid being the most significant, from 8.3% to 24.7%, followed by aspartic acid, from 3.3% to 15.6%.

In the carbohydrate category, reducing sugars increase from 0.7% to 3.16%, sucrose from 0.04% to 3.4%, and glucose from 0.02% to 2%. Reducing sugars increase at the beginning of the fermentation and then decrease gradually due to actions of the salt-tolerant lactic bacteria and yeasts. Also present are xylose and fructose, with fructose present at only detection level.

Changes in pH values are not that significant, between 5.2 and 5.8. However, the total acidity increases during fermentation, from 0.5% to 2.6%. Acetic and lactic acid increase from 0.10% to 0.23%, and 0.11% to 0.23%, respectively. Also detected are citric, malic, oxalic, and succinic acids.

Linoleic and oleic acids increase from 0.19% to 9.65%, and 0.23% to 4.50%, respectively.

Flavor-related compounds are 5 IMP, 5 UMP, and 5 CMP, with 5 IMP making up the largest amount, at 0.06–0.15%. Odor-related compounds include acids, alcohols, phenols, lactones, ketones, esters, aldehydes, furans, pyrans, thio compounds, and nitrogen-containing compounds. Isovaleric acid increases from 365 to 1836 ppb; isoamylalcohol (from leucine) increases from 570 to 11244, phenyl ethyl alcohol (from phenylalanine) increases from 903 to 6836 ppb; n-hexanol increases from 53 to 537 ppb. Other compounds exist only as traces.

When the bean koji making process is reduced to 48 hr, there are higher activity rates of amylase, protease, and lipase. This is especially true for the wet fermentation process with addition of 45% water. The mash has a better hydrolysis rate for proteins and a higher total nitrogen utilization rate. After 3 months of fermentation, there is a higher

protease activity, with subsequently more amino acids, and more deaminase and decarboxylase hydrolysate products such as isoamyl alcohol and phenyl ethyl alcohol. When the fermentation is extended to 5 months, the protein hydrolysis rate is 75%, and total nitrogen utilization rate is 77%. These are higher values as compared to values obtained from traditional methods. Total free amino acid content in wet fermentation is about the same as that in dry fermentation. However, there is less glutamate in wet fermentation with undetectable amounts of glutamate from the dry fermentation. Both fermentation processes produce more arginine.

When the incubation temperature for bean koji making is increased from 45°C for 4 hr to 50–60°C for 2 hr, the incubation time can be reduced. From the viewpoints of free fatty acids, nucleotide, and organic acids, products made from 20°C constant temperature incubation have better flavor. However, from the viewpoints of amylase, protease, and lipase activities, incubation at 45°C for 4 hr shows higher enzymatic activities. When the incubation temperature is raised to 50–60°C, the incubation time can be reduced to 2–3 hr. From the viewpoint of flavor compounds, incubation at 45–50°C followed by sun-drying produce better-flavored products.

9. Product Quality and Packaging

Douchi is a salt-containing, intermediate-moisture, fermented soybean product with storage stability. It contains rich amounts of protein, amino acids, unsaturated fatty acids, and flavor compounds. Its composition is listed in Table 3.

Douchi is packaged before retailing. The packaging can be large or small. Large packages are packed in a small bamboo container (in Taiwan), small packages in plastic bags. They are then wholesaled to retailers. Some douchi from China is also packed in ceramic jars.

V. MISO

A. Introduction

Miso is Japanese fermented soybean paste or thick semisolid product. There are two types of miso, ordinary miso and processed miso. Miso is mainly made from soybean with the addition of enzymes from rice, wheat (barley or rye), or soybean koji and salt. The mixture ferments and ages to become a semisolid-type product. Some call it fermented soybean paste. Based on the koji materials, the ordinary miso can be further separated into rice miso, barley miso, and soybean miso (Table 4). Another classification is based on their

Table 3 Composition of Douchi

	Composition(%)						
Sample	Salt	Acidity	Amino nitrogen (F.N.)	Ammonia nitrogen (A.N.)	Total nitrogen (T.N.)	F.N./ T.N.	A.N./ T.N.
Douchi-mash	12–13	1.1–1.2	1.0–1.1	0.3–0.4	3.1–3.2	30–36	11–14
Filtrate of mash liquid	12–13	1.0–1.1	1.0–1.1	0.3–0.5	2.6–2.7	38–41	19–17

Source: Ref. 5.

Table 4 Miso Formulations and Composition

Materials	Rice miso					Barley miso		Soybean miso
Color/taste	White	Light/ sweet	Light /salty	Red/ sweet	Red/ salty	Sweet	Salty	
Ratio (v/v)								
By soybean	10	10	10	10	10	10	10	10[a]
By rice or wheat	20–25[a]	6.8[a]	6[a]	11–13[a]	5–5.5[a]	5–6[a]	10–12[a]	10
Salt	3–3.5	3.3	4.2	1.8	4.8	4–5	5–7	2–3
Aging time	1–2W	1–2W	2–3W	1–2W	6M	6M	6M	6M
Product components:								
Moisture (%)	43–45	51	49	46	50	46	48	46–49
Protein (%)	8	12	11.3	14	12	10.9	12.5	19–20
Carbohydrate (%)	33	13	13	20	17	15.1	10.3	5
Lipid (%)	2	3.3	5	6.3	5.6	5.8	5	10.3
Salt (%)	5	10.8	13	5.9	13.3	12	12.7	12

[a] Koji-making materials.
W: week; M: month

taste, such as sweet miso, which uses more rice than soybean and less salt, and salty miso, which uses more soybean than rice and more salt. Based on their color, the miso can be classified as white miso (butter color), red miso (reddish brown color), and light-color miso (light yellowish/golden color). If differentiated by their fermentation methods, miso can be classified into natural fermented miso, quick-ripened miso, and nutrient-enriched miso. When judged by product appearance, there is granular miso and chopped miso. Processed miso is the miso with added sugar, oil, or meat.

B. Manufacturing of Miso

A flowchart on miso manufacturing is presented in Fig. 11 (11,12).

1. Raw Material

Soybean, rice or wheat, salt, and water (50% weight of raw materials) are the major components, and seed koji, seasoning and nutritional enrichment ingredients, preservatives, and ethanol are the secondary components.

a. Soybeans Among various soybeans, the yellow-type soybean that is rich in protein (40.3%) and lipid (21%) is suitable for miso processing. The proteins in soybean, rice, and wheat are rich in glutamic acid. The soybean for miso processing in Taiwan comes from the United States. It contains more than 20% lipid with a relatively high percentage of unsaturated fatty acid (more linoleic acid than linolenic acid). There is almost no starch in a mature soybean kernel. Soybean contains 34% carbohydrate, and the polysaccharides are mainly arabinogalactan, units of arabinose and galactose, and the oligosaccharides are composed of 5% sucrose, 3.8% stachyose, 1% raffinose, and a few verbascose. About 7.3% of soybean kernel is seed coats, which contains 12.2% moisture, 8.8% protein, 1.0% lipid, 85.9% carbohydrate, 4.3% ash, 8.7% cold water–soluble material and 13.5% hot water–soluble material. *Comet* and *Kanrich* are the two varieties produced in the United States suitable for miso processing.

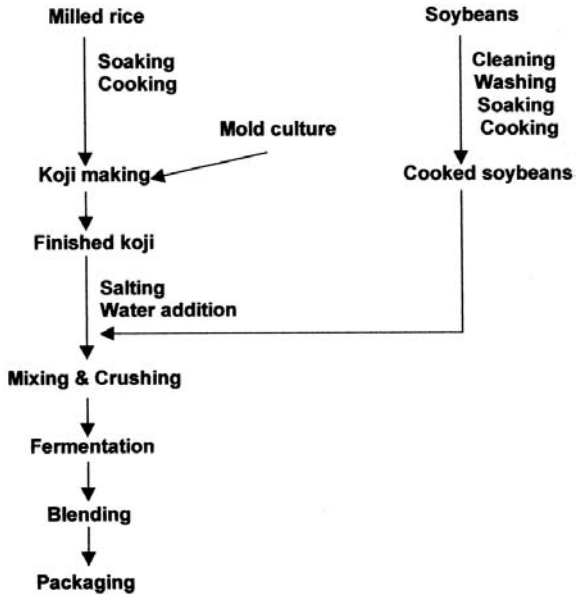

Figure 11 Flowchart on rice miso manufacturing.

The soybean selection criteria for miso are as follows:

1. Easy to cook and soften under mild steam cooking to give lighter color. Extensive cooking may soften soybean; however, it will enhance the color and denature protein excessively. Soybean should absorb 1.3 times the original weight of water before steam cooking. The higher-carbohydrate-content soybean possesses the higher water absorption and water-holding capacity.
2. Bright color after steaming. This is affected by the cooking conditions, temperature, and time. The color of cooked soybean influences the color of the final product. Therefore, soybean with light yellow seed coats, light-colored hilum, and a fresh crop are preferred.
3. Large-kernel soybeans, more than 1000 kernel per 250g.
4. Uniform in variety and size and containing minimal amounts of broken beans or debris.

b. Rice Wetland, nonwaxy, Japonica-type rice is preferred in miso manufacture. It should be polished to 92% (polished white rice). Other types of rice do not possess all the properties required for the making of characteristic miso. The predominant component in rice is carbohydrate, in which starch is primary, with no more than 1% each of dextrin, reducing sugar, and pentosan. The lipids are mainly oleic and linoleic acid in the bran. The important characteristics for rice as raw material for miso making are high moisture uptake, low viscosity, and high rice koji enzymatic activity. The rice koji should be able to dissolve easily and give strong sweet taste and aroma after saccharization. Table 5 lists the proximate composition of rough and polished rice.

c. Wheat Rye is preferred over barley as wheat miso raw material due to the convenience of husk removing. The polish rates for rye and barley are 75–85% and 60–70%, respectively. The proximate composition of wheat and barley is listed in Table 6.

Table 5 Composition of Rough and Polished Rice (%)

	Moisture	Protein	Lipid	Carbohydrate	Fiber	Ash
Rough rice	15.5	7.4	3.0	71.8	1.0	1.3
Polished rice	15.5	7.2	1.7	74.4	0.4	0.8

Source: Ref. 9.

Wheat provides more protein and calcium than rice. Wheat is rich in glutamic acid, which gives stronger umami flavor in wheat miso. Phenol compounds in wheat also provide the unique aroma in wheat miso. Starch is the major component in wheat carbohydate; the others (of which changed to about 8–10%) being araban, xylan, galactoaraban (all pentosans), contributing to the color of wheat miso.

d. Salt Regular coarse salt available on the market (>91% NaCl) is used.

e. Water Drinking-grade potable water is needed because 50% of miso is water.

f. Starter Mold Mature dried spores of *Aspergillus oryzae*, viable count 5–10 $\times$ 10^8/g, are commercially available. The length of hyphae in starter mold can be long, medium or short. Starter mold having medium-length hyphae with sporangiophores (1–3 mm) is used for miso. Short hyphae starter mold can produce stronger protease; long hyphae starter mold produce stronger amylase. As a result, sweet miso uses starter mold with high amylase activity and salty miso uses the one with high protease activity.

2. Raw Material Treatments

a. Handling the Soybeans The soybeans are sorted to remove debris. Removal of the seed coats is only required for finer miso. The beans are washed with and soaked in water at 15°C for 8 hr to allow maximum water absorption. Weight of soaked soybean should increase to 2.2–2.4 times that of raw soybean. The more water the soybean absorbs, the easier it is cooked. However, soybean-type miso will have trouble in the koji-making process if too much water is used. Soaking times of 1–2 hours, 3–5 hours, and 16 hours are recommended for soybean-type miso, red-type miso, and light-color miso, respectively. The soybean for light-color type miso requires a longer time to achieve full moisture absorption. The soaking water needs to be replaced 1–3 times, followed by draining for 1 hr to ensure even moisture distribution, and thus steam can penetrate into the soybean kernel easily.

The purposes of stem-cooking soybean are to denature the protein, inactivate trypsin inhibitor and hemagglutinin, solubilize polysaccharides such as pectin or arabinogalactan, soften soybean texture, remove beany flavor, and eliminate the microorganisms on the surface. There are two cooking methods, steam cooking and boiling, in which less soaking

Table 6 Proximate Composition of Wheat and Barley (%)

	Moisture	Protein	Lipid	Carbohydrate	Fiber	Ash
Barley kernel	14	10	2.8	66.9	3.9	2.4
Wheat kernel	14	10.6	2.8	69.4	1.4	1.8
Polished wheat[a]	13	7.9	1.3	76.8	0.3	0.7

[a] 80% polish rate.
Source: Ref. 9.

time is needed. Soybean that is boiled in water in a 1:4 ratio is easier to get soft and has a lighter color, but more than 10% of the soluble solids could be lost due to dissolution. In ambient steam cooking, it takes 4–8 hours to steam the soybean. However, under 1 kg/cm^2 steam pressure (121°C), it needs only 45–50 min to cook (Figure 12). A rotatory pressure cooker takes only 20–30 min at 0.5–0.7 kg/cm^2 (112–115°C). Recently, a modified continuous steam cooker has been used to cook at 1.5–2.0 kg/cm^2 (127–135°C) for 2–5 min. The hardness of steam-cooked soybean should have breaking force lower than 500 g for a single kernel. Breaking force higher than 800 g results in a coarse-textured product, whereas breaking force of less than 300 g results in incomplete fermentation and a viscous product.

The cooking method influences the color of the products. Products with light color are made from boiled soybean because more water–soluble pigments are removed during boiling. To avoid enhancing the browning after cooking, immediate cooling of the cooked soybean to the temperature suitable for mixing (30°C) is required. Vacuum cooling, cool-air, or conveyer-type coolers are available to cool the soybeans rapidly rather than the old-fashioned method in which the soybeans are spread and cooled outside the cooking chamber. Such a cooling method needs longer time, and the soybeans are vulnerable to contamination with undesired microorganisms.

Cooled soybeans are further chopped to 5–6 mm diameter particle size. The smaller the size, the easier the digestion goes. Nevertheless, particles ground too fine will delay the fermentation process as well.

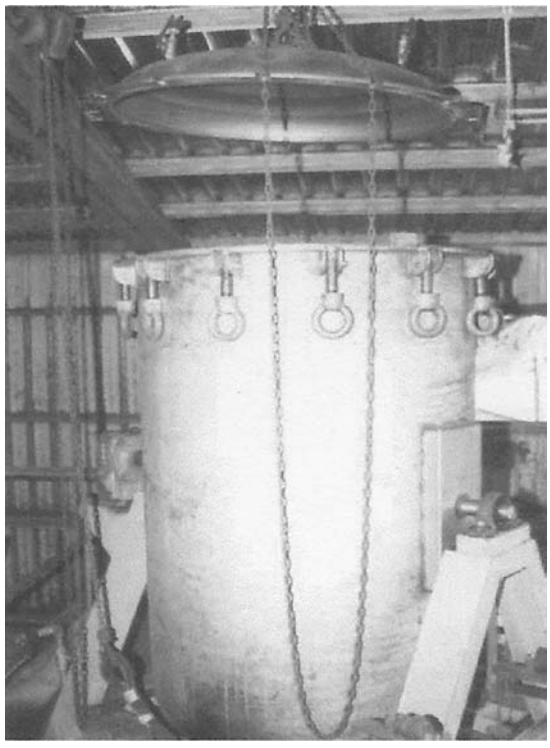

Figure 12 Soybean steamer.

b. Treatment of Rice The degree of rice polishing depends on the type of miso to be processed. Light-color type miso requires rice with a higher degree of polishing (smaller polish rate value), whereas the red-type miso uses rice with lower degree of polishing. The polish rate of rice has to be lower than 93% for proper koji process; 90% is used in current production. The rice proceeds with washing, soaking, and draining steps. The rate of water absorption depends on the texture, polish rate, and water temperature. Rice with soft texture, immaturity, or a higher degree of polishing has a higher absorption rate. Generally, rice is soaked in water for 4 hrs to overnight to allow water to be absorbed evenly in the kernel, and the water absorption rate reaches 28% of original weight for complete starch gelatinization during steam cooking. Too little moisture uptake will produce steamed rice too hard for mycelia to penetrate inside the kernel. Too much moisture uptake will soften the cooked rice, which makes it difficult to process koji.

Rice in the washing and draining stage will lose some water–soluble components and potassium, about 30–50%. Some other ingredients, such as sodium, magnesium, phosphor, sugar, protein, amino acids and lipid will be lost partially while calcium and iron ions will be adsorbed. The purpose of draining are to (a) drain all the surface water to avoid surface stickiness and (b) allow tempering of the moisture. However, prolonged draining time under high temperature will result in rancidity, microbial spoilage, or red discoloration.

Steam cooking is used to gelatinize the starch, to dissolve protein, to help koji mold grow, and to sterilize. If the rice is undercooked, mycelia cannot penetrate inside the ungelatinized kernel resulting in koji with low enzyme activity. Properly cooked rice (a) should be full, elastic, soft inside and hard outside, and without stickiness on the surface and (b) have no white center inside the kernel. Cooked rice will contain 36–37% moisture.

A batch-type steam cooker takes 30–40 min at 1 kg/cm^2 (121°C) to cook the rice properly (Fig. 13). A continuous-type steam cooker uses a stainless conveyer to carry a layer of rice 20 cm thick and simultaneously supplies the stea from the bottom of the conveyer to cook for 25–30 min. For rice with low water absorption or hard texture, the

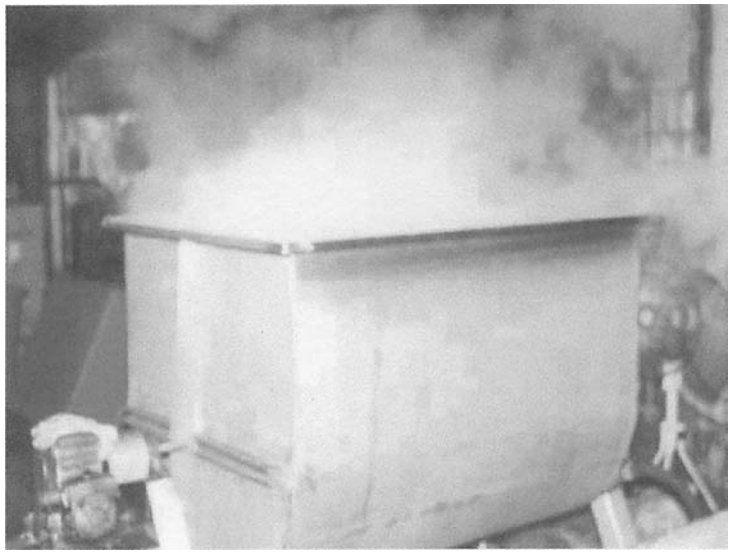

Figure 13 Steam rice.

problem can be solved by steam cooking twice. Cooling can be achieved be either natural cooling or continuous air cooling to the temperature that is ready for koji processing. The temperature depends on the season, koji-processing method, status of koji bed, and the amount of koji piled up for germination. Cooling temperature sets at 3.5°C in the winter and at 3.2°C for the summer to accommodate the subsequent koji process.

c. Handling of Wheat Wheat can absorb moisture faster than rice, and it starts to take up moisture at the beginning of the washing stage. It is important to shorten the soaking time and to prevent uneven moisture distribution. Soaking time should be adjusted along with water temperature used. It takes more than 2 hr to drain the soaked wheat. Drained wheat weighs about 1.28–1.3 times its original weight and has about 35% moisture.

Cooking conditions for both rice and wheat are similar, usually 40–60 min or 30 min under pressurized. Cooked wheat has a light syrup (brown) color and is swollen evenly without surface stickiness. If wheat absorbs too much water or drains incompletely, surface stickiness will occur. Insufficient water absorption can result in ungelatinized white starch particles in the center. Steamed wheat contains 38% moisture.

3. Preparation of Miso Koji

The purposes of koji preparation are as follows:

1. To incubate koji mold to produce the amylase and protease for raw material digestion
2. To incubate koji mold for production of substances that promote growth of salt-tolerant microorganisms and to generate the precursor of miso flavor
3. To allow mycelia penetration into the structure of raw materials and breakdown the structure for enzymes to work
4. To remove raw material off-odor

The optimal temperature for koji mold growth is 30–35°C along with a relative humidity of over 95%. Deviation from this koji temperature range will hinder mold growth and result in a bad koji due to undesirable microorganisms.

Miso koji mold should produce more saccharization-type amylase instead of the liquid-type amylase, especially for white miso or other high koji yield miso. Strong liquid-type amylase in koji mold gives a soft, viscous miso. Insufficient saccharization-type amylase will give a coarse texture with less sweet taste. More amylase is generated with koji produced at high temperature (40°C) than at low temperature (30°C). Therefore, koji mold should be incubated at 35–38°C to have high amylase activity.

Based on optimal pH, there are three types of protease in koji culture: acid (pH 3.0), neutral (pH 6–7), and alkaline (pH 8–10). The protease activity increases as pH value decreases. Acid and neutral proteases affect the protein digestion in miso the most. The neutral protease, the most important one, acts at the mixing step at pH around 6.0. The best temperature for preparing koji with stronger protease activity is 30°C instead of 40°C.

a. Starter Mold Starter mold for miso processes are prepared by multiplying koji mold culture. Barley flake and wheat bran in a 1:1 ratio are mixed with water (50% by weight of raw materials). The mixture is cooked for 30 min under 1 kg/cm^2 pressure (121) followed by cooling to 28–30°C and inoculating with 0.1% long-hyphae koji mold. The starter mold is ready when spore count reaches 8x108/g after incubation at 28–30°C for 3–7 days.

Three to five hours after transferring koji mold, the spores start to germinate and the mycelia grow longer. Although the optimal temperature for fungi growth is 30–35°C after inoculation, the temperature needs to be adjusted to below 30°C to prevent contamination from undesired microorganisms. Mycelia grow actively after 18–34 hours of incubation. Their respiration demands much oxygen and generates CO_2 and heat. It is necessary to supply oxygen and remove the CO_2 as well as to decrease the temperature of starter mold.

b. Traditional Rice Koji Process The traditional rice koji process uses wooden koji trays and incubators. Cooked rice is cooled to 30–35°C and inoculated with the 0.1% starter mold. The well-mixed mixture is then covered with a wet towel and incubated at 30–35°C for 3–4 hr for spores to germinate. After 8–10 hr, the mixture starts to generate heat. The temperature reaches its peak after seeding for 18 hr. In order to prevent further increase in temperature and formation of aggregates, the incubated mixture needs to be broken up and blended four times approximately after seeding for 20 hr. The mixture is dispersed separately on several koji trays to avoid elevating temperature and to decrease CO_2 concentration. Location of the koji trays should also be exchanged to provide even temperature distribution after the second blend. After incubating for 40 hr, the koji does not form additional spores. The koji is ready to be removed and cooled. Finished koji should be mixed with salt as a salted-koji to inhibit further generation of heat and moldy off-odor.

c. Mechanical Koji Process Compared with the traditional method, the mechanical koji process saves a lot time and labor. In recent years, the mechanical method has commonly been applied to replace the manual blending procedure. Air with proper temperature and relative humidity is introduced to pass through the surface or the center of the koji mixture and to exhaust the generated heat for temperature maintenance of the mixture. Two ventilation types are available for mechanical koji process: surface type and inner type. There are four inner types (steady-bed, shed, rotatory-drum, and high-pile) depending on the position of koji tray placed. Mechanical ventilation controls the temperature easier by maintaining vented air at 30–32°C with minimal ventilation. After 28–30 hr of incubation, the piled mixture starts to generate a large quantity of heat. The mycelia lengthen and tangle with each other, making the ventilation difficult. Then manual blending is needed due to poor temperature adjustment. Saturated humid air with temperature adjusted to below 35°C is supplied until 2–3 hr before finished koji is ready.

d. Wheat Koji Wheat generates more heat than rice due to the higher content of protein, mineral, and vitamins. Koji mold will not be able to grow due to easier moisture evaporation on the wheat surface. High humidity should be maintained at the beginning of koji preparation. Ventilation of humid air should be coordinated with the corresponding temperature of koji in mechanical koji preparation. In order to have a koji with strong amylase, lighter color, and a high production rate, we need to control the temperature at 36–38°C for the first 10–18 hr, followed by adjustment to 30–32°C. The koji for red-type miso has high protease and amylase activities, and the koji temperature is controlled at 29–31°C. With its high moisture content and stickiness, wheat koji is more easily contaminated by *Neurospora* than rice koji.

e. Soybean Koji Steam-cooked soybean requires mechanical kneading into a miso (soybean) ball at 60°C. With 50% water content, steam-cooked soybean is prone to *B. subtilis* contamination. Facultative anaerobic lactic acid bacteria grow inside the soybean ball. This lowers the pH to prevent *B. subtilis* growth, and the soybean ball becomes a suitable environment for fungi to multiply safely. The size of the soybean ball depends on the size of the device available, with a large ball more than 45 mm in diameter and the small ball 19–24 mm in diameter. Small soybean balls are commonly used for miso making.

Fungi growing on the surface or surface layer of large soybean balls result in less enzyme activity. Because lactic acid bacteria can grow inside the soybean ball, more lactic acid is obtained from a larger ball. A small soybean ball has a faster aging rate and a darker color than a large ball. It is important to avoid high temperature at the early stage of soybean koji preparation and to blend the moist soybean ball with starter mold thoroughly to control possible *B. subtilis* contamination.

f. Storage of Prepared Finished Koji To prevent further heat generation, the finished koji is mixed immediately with one-third of the total salt required for the formulation. Normally, the salted koji should be used in 2 days due to gradual loss of enzyme activity.

The criteria for a good finished koji are as follows:

1. Possession of proper enzyme activity
2. No contamination from undesirable microorganisms
3. Good mycelial growth into all rice kernels
4. Bright and without browning in appearance
5. Possession of miso aroma without off-odor
6. Elastic and soft to handfeel

Depending on the types of miso, the level of each criterion may be varied. For example, light-color miso requires no browning in appearance and high amylase activity, whereas red-color salty-type miso needs high protease activity.

4. Mixing

While the cooked soybean is cooled, salted koji, salt and sterilized brine with some yeast, lactic acid bacteria, and vitamins are added. Then the mixture is chopped through a 6 mm (for granular-type miso) or 1–2 mm (for fine-type miso) particle size sieve plate and put into a fermentation container for further fermentation and aging (Fig. 14). Barrel-type mixer (Fig. 15), automatical weighing mixer, screw-type mixer, or rotary drum–type mixer are used for large-quantity of mixture; the small quantity can be mixed in a tank manually.

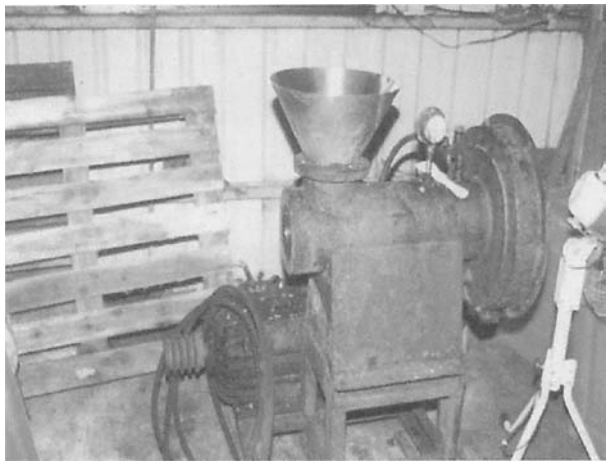

Figure 14 Crusher of materials for miso making.

Figure 15 Barrel mixer.

Light-color type miso requires the salted koji mixed with cooked soybean while the cooked soybean is still warm. The key factor in mixing is thorough mixing. The salt concentration should not deviate for more than 0.5% in the mixed mash. The temperature of mixed mash, an influencing factor for the aging process, will affect its enzyme activity, microbial growth, or chemical reaction. For sweet-type miso, mixing is carried out at 50°C to inhibit microbial growth and to pursue high enzyme activity. If the mixing temperature is below 30 C, the aging process will be in trouble. Salty-type miso requires fermented aroma and therefore is mixed at 28–32°C in warm fermentation method or at 20°C in natural fermentation method (Fig. 16). In general, adjustment of the mixing temperature relies on the temperature of the cooked soybeans. The end temperature of the mixture, except for the high temperature mixing, is usually maintained at 20–25°C, or even at 10°C, which gives better-quality miso.

Addition of sterilized water can adjust the water content and the texture of miso. It also assists the fermentation and aging processes. The amount of water needed depends on the moisture of cooked soybean and miso koji. It can be calculated as follows:

$$\text{Amount of sterilized water} = \text{Total amount in mixing} - (\text{Cooked soybean} + \text{finished koji} + \text{salt})$$

$$\text{Amount of miso mixture} = \frac{(\text{Total solid of cooked soybean, finished koji and salt})}{(100 - \text{added water percentage by total weight})} \times 100$$

The amount of moisture can affect the microbial growth or aging process even though the mixture have the same amount of salt. The relationship between the moisture and the amount of salt can be expressed as follows:

$$\text{Concentration of salt by total water}(\%) = \frac{\text{Salt}(\%)}{\text{Moisture in miso}(\%) + \text{salt}(\%)} \times 100$$

Figure 16 Fermentation of miso.

The concentration of salt by total water in salty miso is 21–22% when soybean and koji are in a 1: 5–7 ratio and it is 20–21% when koji ratio is 8–10.

A clean plastic cloth is used to cover the top of the fermentation tank, and a piece of rock 20–30% of total mixture weight on top of it ensures that the miso is under anaerobic condition. The rock weight is usually heavy to press out enough fermentation liquid for covering the surface of the miso.

The salt-tolerant microorganisms used in the mixture for enhancing the fermentation can be yeast such as *Saccharomyces rouxii*, *Torulopsis versatilis*, and the lactic acid–producing bacteria *Pediococcus halophilus*. Antagonism occurs between yeast and lactic acid bacteria, and the growth of lactic acid bacteria can be hindered by yeast. Therefore, the amount of lactic acid bacteria used (10^6 cell/g) should be 10 times higher than the yeast (10^5 cell/g).

5. Fermentation Management

The purposes of miso aging are as follows:

1. To utilize enzymes from miso koji to disintegrate the raw materials, and
2. To utilize the fermentation power of salt-tolerant microorganisms (yeast and lactic acid–producing bacteria) in which chemical degradation or synthesis of fermented products can generate color as well as flavor at early stage of fermentation and aging.

After mixing, the first stage of fermentation, primarily enzymatic reactions, lasts for about 10 days and the environment is suitable for growth of salt-tolerant microorganisms. The optimal temperature for the digestion of protein and carbohydrates by the enzymes from miso koji are 45–50°C for protease, and 55–60°C for amylase. Therefore, sweet-type miso, which relies on enzymatic reactions rather than fermentation, has optimal temperature around 55–60°C. However, the aging process at such a high temperature kills yeast and lactic acid bacteria and causes dark color and burnt off-flavor.

Some microorganisms grow in miso koji and the surrounding environment, and are added microorganisms for fermentation. Most of these microorganisms are not salt-

tolerant, cannot grow under the 20% concentration of brine and will die out gradually in a week. Then the remaining salt-tolerant microorganisms begin to grow. For those desirable yeast and lactic acid bacteria in miso, the optimal temperature is about 30°C they stop growing at 40°C.

A salty-type miso requires both enzymatic reaction and fermentation. The optimal temperature for the microorganism growth and aging is about 30°C. For example, a fermentation for salty-type miso containing 12–13% salt should be mixed at 25–30°C and maintained at this temperature for 7–15 days for lactic acid bacteria to grow and for lowering the pH below 5.5, and to build an suitable environment for the yeasts. *Saccharomyces rouxii* produces some flavor components such as ethyl alcohol and amyl alcohol. *Torulopsis versatilis* can convert lignin into ferulaic acid and further into 4-ethylguaiacol, a flavor component. During this period, the temperature of miso rises slowly to 30–35°C. Temperature is maintained for 15–30 days, followed by a decrease in miso temperature to 20–25°C for 40–60 days for post-aging. The post-aging process is carried out under lower temperatures to allow slow miso color development and to temper all the flavor components. When a balanced flavor is developed from the blending of sweet taste from sugars, salty taste from salt, acid taste from organic acids, and umami taste from amino acids, the aging is complete. A browning reaction from amino carbonyl reaction is also developed simultaneously. The control points for fermentation are the temperature programming as well as blending technique, frequency, and schedule.

Natural fermentation of miso does not apply any thermal treatment nor temperature control, but sometimes long-term aging proceeds in the manually adjustable fermentation room. Blending procedure can assure homogeneous fermentation. At least one blending is required after 2–3 weeks of mixing due to vigorous fermentation, and later another one or two blendings can be helpful. Blending can evenly maintain the temperature of the mixture and homogenize the mixture, as well as supply the oxygen for yeast to grow.

The quality of miso can be evaluated using a sensory method or chemical determination. Color can be judged visually or determined by colorimeter. The color brightness decreases and redness increases as the aging proceeds. Acidity I is the freshness-related acid determination. The pH value of raw materials at an early stage of mixing is 5.7–5.8. Protein disintegration produces amino acids and organic acids, which increases titratable acidity I. The pH values become 4.9–5.1 at the end of aging. Protein solubility is defined as the ratio of total nitrogen and water–soluble nitrogen, and protein digestibility is defined as the ratio of total nitrogen and formol (amino group nitrogen) nitrogen. The higher the degree of aging process, the higher the values of protein solubility and digestibility are determined. Both values increase significantly at early stage of aging. The values stabilized 30–40 days from the beginning of aging. After 50 days, protein solubility and protein digestibility are about 60% and 25%, respectively. Free amino acids (glutamic acid and aspartic acid) can also be measured. Alcohol and lactic acid contents vary among different types of miso. Alcohol and lactic acid content should be higher than 0.2% in yeast-related fermentation. If the values are below 0.05–0.1%, there are no active yeast or lactic acid bacteria. Free fatty acid comes from the lipid in soybean, which is hydrolyzed by lipases from starter mold. Some fatty acids form lipo-ester components contributing to miso flavor. Therefore, the determination of fatty acids or lipo-esters becomes the index of aging.

6. Product Standardization

For quality consistence, blending is needed after aging. Granular texture–type miso can be used for blending directly, but fine texture–type miso needs chopping through a 1–2 mm

sieve. A too-small-diameter sieve or a slow-speed chopper may cause sticky product. When preservative is needed, we can add sorbic acid (less than 0.1%) or K-sorbate (0.05%). Alcohol (2–3%) can also be used as a substitute preservative. The level of residual alcohol in miso can be determined to estimate the amount of alcohol needed for addition. It is very important to blend the alcohol thoroughly. Sterilizing the aged product in hot water can also preserve the miso. The yeasts in raw miso cause continuous fermentation after bottling. In small operation, miso is packed in small packs, sealed, and sterilized at 60°C for 10 min or 70°C for 5 min. For continuous sterilization and packaging, miso is heated and cooled in pumping pipes followed by packaging. However, this method is not done under strictly aseptic conditions, and additional care is needed to prevent contamination.

7. Packaging

Single polyethylene bags were first used for small packs of miso. Now, laminated films such as cellophane and polyethylene coated with vinyliden chloride or laminate poly-cellophane are available. In order to maintain quality, film with low gas permeability, which can slow down the browning of product, is recommended. Plastic containers are also available.

There are manual and automatic filler packaging machines. Before sealing the package, we should remove the air to prevent rapid browning. Product stored under room temperature will undergo color and flavor deterioration. If miso is stored at high temperature, severe browning occurs, and miso pH continues to drop with increases in titratable acidity, resulting in a browning and off-flavor. Low temperature storage (lower than 15°C) can prohibit such deterioration.

C. Product Quality

Good-quality miso can be described as having (1) unique aroma without off-flavor, (2) light sweetness and no odd-odor or sour taste, (3) even mycelial growth on each rice kernel without undesired microorganisms, and (4) soft but elastic texture with proper adhesiveness of rice kernels. A good miso product must be free from off-flavor (sour flavor) or alcohol flavor, mold infection, and discoloration (3).

By Republic of China National Standard (10), miso should meet the following criteria: (1) possesses proper miso aroma, (2) total nitrogen is higher than 1.51, (3) nonsalt solid content is higher than 40.80%, (4) pH ranges from 4.85 to 5.85, (5) packaging material should be clean and waterproof.

VI. DOU-PAN CHIANG (OR TOU-PAN CHIANG)

A. Introduction

Dou-pan chiang is a traditional Chinese food. It is manufactured in various places in China. Traditionally, it was made mostly from *Vicia faba* (faba beans, horse beans, or wing beans). However, with the easy access to soybeans, it is now also commonly manufactured using soybeans, such as is done in Taiwan, using similar procedures.

Besides soybeans, dou-pan-chiang also uses wheat, brine, and starter as the major ingredients. Various seasonings such as chili pepper, pepper, fennel, and anise seeds can be added to modify the flavor to meet consumer demands. Its nutritional composition is similar to miso. This section discusses the handling of raw materials, manufacturing process, and changes during fermentation in the making of dou-pan-chiang.

B. Manufacturing of Dou-Pan-Chiang

The flowchart of dou-pan-chiang manufacturing is presented in Fig. 17.

1. Raw Material Handling

a. Soybeans Handling of the soybeans for making dou-pan chiang follows the same procedure for making soy sauce (see Chapter 29). The cooked soybeans are mixed with roasted (or pan-fried) wheat at a ratio of 10:2 (w/w) for the koji starter preparation. The wheat is in a wheat flour form instead of broken pieces (grits). Other steps are the same as in the making of soy sauce koji starter.

b. Wheat Flour The wheat flour is browned in a frying pan or roasted to light brownish yellow color.

c. Brine Commercial crude salt is used to prepare a brine of 20–21° Baume21.18–22.24% salt) at 15°C.

2. Preparation of Seed Koji (Starter)

In the past, the seed koji (inoculum) came from the leftover Dou-pan-chiang from the previous making. It was mixed with the medium to prepare the starter. With the availability of pure cultures, this traditional method is no longer used. The current method is to use slightly (1–2%) polished rice as the raw material. It is washed, soaked, steamed, and cooled to 40°C or lower, followed by mixing with 2% potash made from

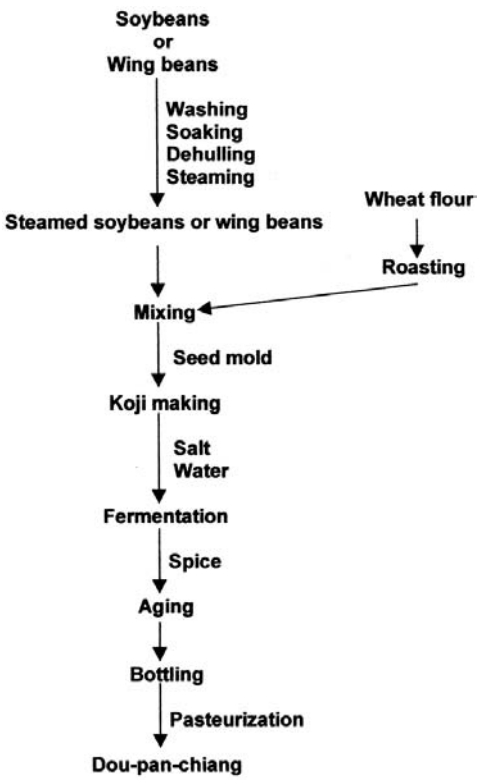

Figure 17 Flowchart on Dou-pan chiang manufacturing.

wide-leaf wood and 1–2% (w/w) dou-pan chiang koji starter (seed koji) or 1–2 $\times$ 10^8/g spores of the starter (*Aspergillus oryzae* or *A. sojae*).

The purpose of adding the potash is to

1. Provide inorganic nutrients for the formation of the spores
2. Provide an alkaline environment to inhibit undesirable microorganisms
3. Adjust the moisture content of the mixture so that the mixture will not stick together and has better aeration
4. Extend the storage stability of the spores

The koji starter is incubated at 25–30°C for 5–6 days in the incubator room, taken out from the incubator room, and sun-dried or mechanically dried to contain 5–8% moisture. It is then weighed and packed for marketing or can be used directly for the making of dou-pan-chiang. Commercially, the dou-pan-chiang koji starter either can be granules of the koji starter or, if has been sieved, contains only the starter powder of the spores.

3. Preparation of the Koji Starter

Preparation of the koji starter is basically the same as the preparation of koji starter for soy sauce making (see Chapter 29). In the preparation of dou-pan-chiang koji starter, there are other microorganisms such as bacteria and yeasts from the environment besides the inoculated *A. oryzae*. In each gram of the koji starter, the total bacterial count, mold (spore) count, yeast count, and lacto-bacteria count are about 10^7–10^9, 10^7–10^8, 10^4–10^5, and 10^4–10^5, respectively. The environmental requirements determine the microbial counts and changes in the kinds of microorganisms. If the foreign microbial counts are too high, they will affect the growth of the koji mold, and directly influence the utilization rate of raw materials, product quality, and product stability. Therefore, it is important that the environment be kept sanitary to avoid unnecessary contamination.

In the preparation of Dou-pan chiang koji starter, with incubation of *A. oryzae* at 30°C for 72 hr, the activity of neutral protease (related to the hydrolysis of nitrogenous compounds) reaches 2397–3688 units/g dry matter. The activity of neutral protease is highest after 48-hr incubation. At 60-hr incubation, the acid protease activity is highest. The α-amylase activity increases gradually with incubation time. The activity of β-amylase is highest at 23–60 hr. The activities of α-galactosidase, cellulase, and lipase reach their peak at 30–60 hr. The pH value as well as amino nitrogen, ammonia nitrogen, and free fatty acids contents increase gradually with incubation time. Moisture and crude fat contents decrease gradually. However, no significant change in total nitrogen was observed. Total and reducing sugars increase at the beginning and then decrease afterwards.

4. Making of Dou-Pan-Chiang

The Dou-pan-chiang koji starter is mixed with 20–21°Baume (21.18–22.24% salt) brine at a ratio of 1:1 (w/w) in tubs. These tubs are left outdoors for solar heating and stirred with a wooden paddle once every day. They are covered at night or when it is raining. In the winter, supplemental heat insulation may be considered. Depending on the intermediate product temperature, the fermentation may take 2–5 months.

When addition of seasoning such as chili pepper or pepper is desired, it can be added at the end of fermentation in the form of powder or crushed seasoning (Fig. 18). Chili pepper can also be added in form of chili pepper oil made by heating seedless chili pepper in peanut oil. Addition of 20–40% cooked soybean at the time of packing into jars will

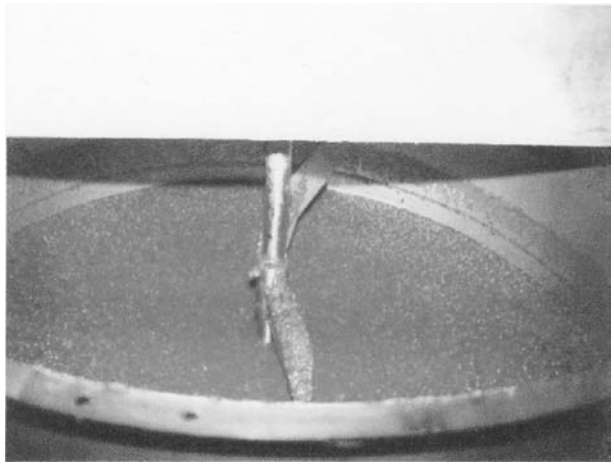

Figure 18 Mixing of Dou-Pan chiang.

increase the flavor. Dou-pan chiang is usually packed in ceramic or glass jars (Figs. 19, 20). The jars have to be heat-sterilized in boiling water for about 20 min and are then capped and sealed.

C. Changes During Fermentation (13)

1. Changes in Microbial Population During Fermentation

At the initial stage of fermentation, the mold count decreases rapidly. More salt-tolerant lactic acid bacteria and fewer yeast increase accordingly. As fermentation progresses, lactic

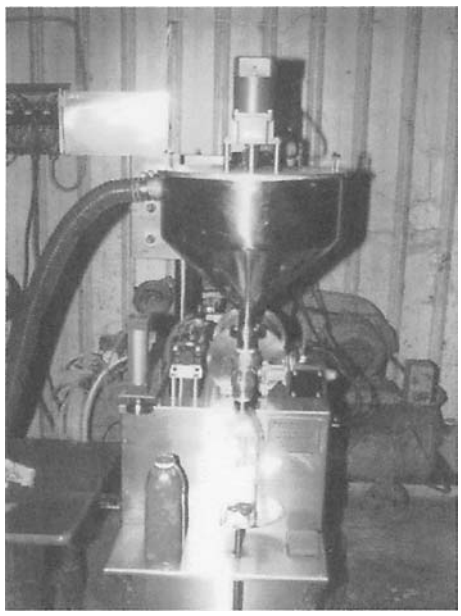

Figure 19 Bottling machine for Dou-pan chiang.

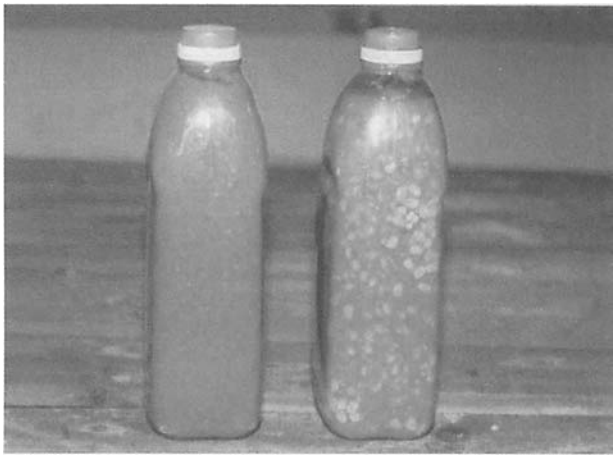

Figure 20 Bottled Dou-pan chiang.

acid bacteria and yeasts continue to increase, and eventually there are more yeasts than lactic acid bacteria. The lactic acid bacteria include *Pediococcus*, *Streptococcus*, and *Tetracoccus* sp. Yeasts include *Candida*, *Cryptococcus*, *Kluyveromyces*, *Rhodotorula*, *Saccharomyces*, and *Torulopsis*. They all contribute to the flavor of Dou-pan-chiang.

2. Changes in Enzymatic Activities During Fermentation

At the beginning of fermentation, acid protease has higher activity. Neutral protease has higher activity at the end—about 40%, compared to acid protease activity decrease to only about 20%.

At the initial stage of fermentation, both α- and β-amylase activities increase gradually. With progression of fermentation, they decrease gradually. At the end of the fermentation, α-amylase retains about 40% activity, whereas β-amylase retains 60% activity. The amylase demonstrated higher salt-tolerance than protease.

Cellulase stays at about 35% activity at the beginning and at the end of the fermentation. Its salt tolerance is somewhat between that of amylase and protease.

The activity of α-galactosidase increases slightly at the beginning and then decreases afterwards, with only about 5% activity at the end of fermentation. It is not very salt-tolerant.

Lipase activity increases slightly at the beginning followed by a rapid decrease, and then again increases. This kind of enzymatic change shows that the lipase from the koji starter is not salt-tolerant, and the lipase from yeasts is more salt-tolerant.

3. Changes in pH, Acidity, and Alcohol Content

During the maturation period of Dou-pan-chiang fermentation, the salt-tolerant micro-organisms produce organic acids. The koji enzymes hydrolyze the substrates into free fatty acids and amino acids. The autolysis of the microbial cells produces the nucleotides. With all the acids produced, the pH gradually drops to about 5.4, and the total acidity gradually increases. The production of alcohol is irregular at the beginning, and in the mid-fermentation period increases to about 0.45% due to an increase in the yeast population.

At the end of fermentation, alcohol decreases due to reactions with free fatty acids and organic acid to form the volatile flavoring compounds.

4. Changes in Color Intensity and Soluble Solids

The color of Dou-pan-chiang changes with the progression of fermentation. At the initial stage of fermentation, the color brightness increases slightly, and then decreases with the progression of fermentation. The color changes from slightly green to slightly red with an increase in yellowness, then to a dull brown color. These color changes are caused by factors such as the temperature, pH value, iron ions, amino nitrogen and reducing sugar contents, time, polyphenol oxidase activity, heating, and enzymatic and nonenzymatic browning reactions.

The soluble solids content in the fermentation mixture increases during the maturation period. This is due to the breakdown of the large molecules to small molecules during fermentation.

5. Changes in Selected Chemical Composition of the Fermentation Mixture

Total nitrogen content of the fermentation mixture increases at the initial stage of fermentation, followed by gradual decrease with progression of fermentation. The amino nitrogen and ammonia nitrogen contents increase gradually during fermentation with a more stable situation at the later period. Proteins in the raw materials are first hydrolyzed by the endoprotease into peptides, followed by the exoprotease hydrolysis to amino acids. The deaminase and decarboxylase then hydrolyze the amino acids to ammonia and amines.

The reducing sugars of the fermentation mixture increase at the initial stage and then gradually decrease.

Crude fat and free fatty acids in the fermentation mixture increase slightly at the initial stage of fermentation, followed by a gradual decrease as fermentation progresses. This is because free fatty acids, produced in the initial stage of fermentation, react with the alcohol produced to form esters.

Organic acids such as acetic acid, lactic acid, oxalic acid, and succinic acid increase with fermentation. This is due to activities of the lactic acid bacteria and yeasts.

Nucleotides such as 5′-UMP, 5′-IMP and 5′-GMP are produced during dou-pan-chiang fermentation. Both 5′-IMP and 5′-GMP showed contents above their taste threshold levels (0.012% for 5′-IMP and 0.0035% for 5′-GMP), with 5 IMP at 0.1 mg/g dry matter and 5′-GMP at 2.1 mg/g dry matter after 96 days. The production of these nucleotides contributes partially to the umami taste of Dou-pan-chiang.

The essential amino acid leucine increases threefold during dou-pan-chiang fermentation. Glutamic acid increases fourfold during the fermentation.

D. Quality Aspect of Dou-Pan-Chiang

Dou-pan-chiang contains various easily absorbable amino acids, reducing sugars, fatty acids, organic acids, and nucleotides that contribute to its characteristic flavor. The flavor of this fermented soy product is well accepted by the consumers. It is usually available in sterilized ceramic or glass jars.

According to the Republic of China National Standards (CNS 611) (14), dou-pan-chiang should meet the following requirements:

1. Outer appearance: easily recognized bean cotyledons
2. Color: characteristic brownish yellow color

3. Odor: characteristic fermented dou-pan-chiang odor, absence of caramel odor, furfural odor, and other undesirable odors
4. Water: total water and volatile content less than 70% (wt. basis)
5. Salt: between 10 and 18% (wt. basis)
6. Crude protein at least 8% (wt. basis)
7. Ash: not more than 19%, including salt
8. Foreign matter: contaminants and foreign matter not permitted
9. Labeling: must meet CNS 3192 food packaging requirements

VII. SANITATION MANAGEMENT AND QUALITY CONTROLS

A. Sanitary Management

Fermented soy foods are traditional, fermented foods with characteristic flavor produced by utilizing the enzymatic reactions of microorganisms and suitable processing methods. In the manufacturing processes, sanitary management is critical. Besides those criteria that should be followed as discussed in the chapter on soy sauce, suitable microorganisms have to be selected for the various kinds of fermented soy products for proper fermentation. During the course of manufacture, it is critical to avoid contamination with poisonous and pathogenic microorganisms that may originate from the mouths or digestive tracts of workers. Prevention of attacks by rodents, flies, roaches, ticks, and parasites is also essential. Contamination from poisonous chemicals, agricultural chemical residues, and radioactive substances must be prevented. Application of food additives must follow regulations governing the use of these additives. At the same time, toxic compounds that may leach out from packaging materials and containers must be properly prevented. Product must be properly stored and retailed under sanitary conditions to ensure food safety.

B. Quality Control

Besides those control measures discussed in the chapter on soy sauce, quality control of fermented soy products must meet compositional criteria. In general, fermented soy products have their characteristic flavors (odors and taste). Quality changes in these products are mainly due to changes in their chemical components, physical state, and texture. Therefore, the quality of these products should not change if these components remain unchanged. However, changes do occur due to the following causes:

1. Attack by organisms such as rodents, flies, roaches, and ticks and contamination from bacteria, molds, and yeasts.
2. Enzymatic reactions caused by enzymes from the foods themselves or from contaminated microorganisms.
3. Oxygen. When oxygen in the environment dissolves in the liquid of the food or is in contact with the surface of the food, quality changes in these products do occur.
4. Reactions with very reactive substances in the foods themselves, such as unsaturated double bonds, or carbonyl compounds that cause color change, most noticeably browning.

In fact, many quality changes in foods are due to one or more of the abovementioned causes. In order to prevent such quality changes, effective methods include low-

temperature storage, low moisture content with water activity (A_w) below 0.35, and exclusion of oxygen. If these criteria are met, changes in food due to chemical reactions can be reduced. The effects of temperature and moisture content are particularly significant.

Good quality natto should have uniform, large-sized beans. The natto beans should be covered with the bacterial slime with luster. They should stick together to form a soft block. The mucilage should be strongly sticky and can form long threads. Natto beans should possess a characteristic odor and be free of offensive odor.

A good-quality hamanatto product should have dark-brown color and strong umami taste. The moisture, protein, lipid, sugar, ash and fiber content of Hamanatto are 36–38%, 26%, 12%, 6–7%, 12%, and 2.9%, respectively.

Good-quality Douchi has a unique aroma and no off-odor or sour or bitter taste.

Good-quality miso has bright and sticky maltose-like color. A pungent salty taste in a sample results from improper mixing of the bean koji and salt or inadequate fermentation (maturation). Another procedure for tasting quality is to add hot water to a small sample of miso followed by stirring. Good miso dissolves easily and forms a cloudy liquid that does not form sediment easily. If the sample shows separation of liquid and sediment immediately, that is an indication of poor quality. It is improper to add water to miso to increase the volume. Therefore, a soft miso is not considered acceptable. At the same time, it is important not to allow the growth of maggots or the presence of rodent droppings.

Good quality dou-pan-chiang should have cotyledon-shaped pieces of a yellowish brown color and a characteristic odor from fermentation. They should be free of undesirable burnt caramel or furfural odor and sour or bitter taste.

Basic quality control and sanitation, and their management, are the responsibilities of the producers, retailers, and consumers. These principles have to be followed to ensure food safety.

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Fermented Tofu: Sufu and Stinky Tofu

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I. INTRODUCTION

Sufu (Chinese soy cheese) and stinky tofu (chaotofu) are block-type fermented soy foods. In the manufacture of these fermented foods, sufu utilizes mainly mold(s), whereas stinky tofu utilizes mainly bacteria. Molds multiply under aerobic condition and produce amylase, protease, and other hydrolases. These enzymes then hydrolyze their substrates. However, molds do not grow under anaerobic conditions but can use the enzymes they produced to hydrolyze their substrates. Therefore, under aerobic conditions, molds metabolize through respiration, whereas under anaerobic condition, they conduct anaerobic respiration and metabolize through fermentation. Using molds to conduct fermentation is a combined utilization of these enzymatic reactions to process food.

In the manufacture of traditionally fermented foods by the so-called traditional fermentation processes, molds and bacteria come from the spores present naturally in the raw materials, in the air in the manufacturing environment, or on the utensils. These traditionally produced products from this household-type industry have difficulty in expanding their production to meet market demand. In order to stabilize product quality, it is therefore necessary to apply specific molds and bacteria through pure culturing techniques. The fermentation technique is then transformed from natural fermentation to pure culture fermentation.

A bacterium is much smaller than a mold, and bacteria are not as complicated as yeasts or molds. Even though some of bacteria have similar shapes, their functions can be significantly different. Morphologically, bacteria may have the same shape, but they can be either beneficial or harmful. It is therefore necessary to understand their physiological characteristics.

The physiological characteristics of bacteria are quite complicated. Bacteria may be classified as aerobic, anaerobic, psychrophilic, thermophilic, and nutrient-selective. Their products are also very complicated.

Some bacteria produce spores, and others do not. In general, bacteria that produce endogenous spores are more harmful. The bacterial cells themselves protect these spores. They are heat-resistant during thermal sterilization. A temperature of 110–120°C for 3–5 min under pressurized steam is needed to kill these spores. Under normal pressure and at 100°C, non-sporeforming bacteria cells can be killed easily, but these spores can withstand 30 min under the same temperature condition without much killing effect. Therefore, it is

necessary to wait for the spores to germinate and then apply an intermittent sterilization process to inactivate them.

When bacteria are utilized during fermentation, their growth is not easily observable compared to molds. Usually, we have to observe physical changes in the substrate such as hardness and mucilage production, or sensory changes in color, odor, and taste, in order to understand the bacterial growth condition.

In the fermentation industry, we utilize microbes obtained from pure culture isolation techniques. In principle, the whole fermentation process should be maintained under pure culturing condition. However, in the manufacture of fermented foods, the pure culturing condition is not as critically controlled as in the microbiology laboratory. This is especially true for mold-ripened foods. Mold is used to start the process and is commonly followed by yeast and bacteria. These bacteria and yeasts have a close relationship to the characteristics of the final product. Even though the presence of some bacteria is considered beneficial, their effects on product quality are not well understood. They are considered nonfunctional microorganisms. However, in the case of multi-microbial conditions, these microbes must provide some functions and warrant future investigation.

II. SUFU

A. Introduction

Sufu is one of the traditional soy foods made by fermentation in China and Taiwan. Soybean, the major raw material, is processed into soymilk and then to soybean curd (tofu). Tofu cubes are inoculated with fungi and fermented until they are covered with fungous mycelia. Subsequently, they are soaked in brine (or salt is added to partially dried tofu) and aged in the mashes from wine, miso, or soy sauce. Sufu has a smooth and sticky texture as well as salty taste and is also known as Chinese soybean cheese or vegetable cheese. Sufu is also called stinky tofu and has other names in different regions.

According to the historical records, tofu was first made by Liu An around 179 B.C.–122 B.C. by adding gypsum to soymilk. Around 1500 years ago, a piece of salted and dried tofu was soaked in the soy sauce mash to make the earliest sufu in history. In 1590, sufu was called ream cake in the Japanese language of the Chinese Herbal Encyclopedia. Homemade sufu is very common in Chinese households located along central and southern coast provinces of mainland China and Taiwan. Nowadays, it is sold in jars manufactured by the industry.

In the early 1920s, a sufu market survey in Shan-Hai (or Shanghai) showed several different processing techniques used by Chinese people. By the existence of fungi, type of fungi, and mash, sufu can be classified as follows:

1. No fungi added in soaking and aging
    a. Jen-Nin tofu: predried tofu aged in salt and miso or soy sauce mash
    b. Sufu: predried tofu aged in the salt and koji
2. Fungous mycelia allowed to grow on tofu, followed by soaking in brine and aging in the mash
    a. Jiang sufu: Soaked in brine and aged in miso or soy sauce mash
    b. Red mash sufu: Soaked in brine and aged in a mixture of soy sauce mash and red koji from *Monascus*
    c. Wine mash sufu: Soaked in brine and aged in rice wine mash

d. Red sufu: Soaked in brine and aged in red rice wine mash
e. Flavored sufu: Soaked in brine and aged in the mash that contains olive leaf or aromatic mushroom
f. Sake sufu: Soaked in brine and aged in the mash that contains rice sake
g. Kwantung sufu: Similar to red mash sufu with addition of hot pepper, anise, and xanthoxylon seeds
h. Rose sufu: Similar to red mash sufu with addition of rose mash

Sometimes meat, crab, shrimp or sesame seed are added to produce moldy tofu. Mash formulated with alcohol, salt, and spice can also be used for sufu soak-in-brine and aging processes. Sesame oil or food-grade paraffin can also be used to cover the top of a jar to prevent air contact for better preservation.

B. Making of Sufu

A flowchart for sufu manufacturing is presented in Fig. 1.

Medium-size soybean with yellow or white hilum, thin seed coat that are hard in texture, high in soluble protein and low in fat content are the most suitable for manufacturing sufu.

Processing steps of sufu manufacturing (Fig. 1) can be divided into four stages. The first stage is to process soybeans to tofu cubes (1 day). The second stage is to inoculate the tofu cubes with fungi and allow mycelia to cover the tofu cube surface (3 days). The third

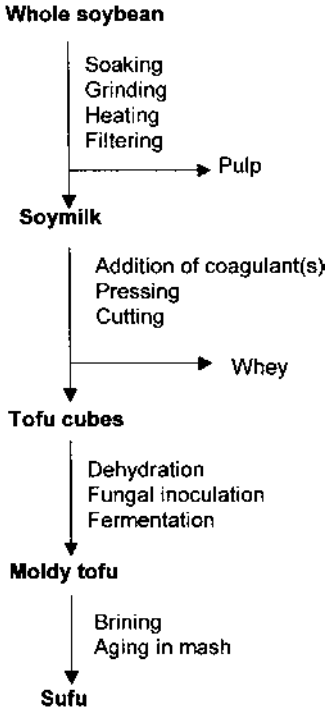

Figure 1 Flowchart for sufu manufacturing. (From Ref. 1.)

step is to allow moldy tofu cubes to dry with spreading of salt onto it (or brining) followed by thermal dehydration to harden the moldy tofu cubes. The last step is to soak and age the moldy tofu cubes in the mash.

1. Preparation of Soymilk and Making of Tofu

a. Preparation of Soymilk Soybeans are processed into soymilk and then into tofu. Fully hydrate the soybean in water until their weight reaches 2.2 times the original soybean weight. Soaking time depends on the temperature of water used: 10°C for 18 hr, 20°C for 10 hr, or 30°C for 6 hr. Soaked soybeans and water in a 1:2 or 1:4 ratio is then disintegrated with a grinder. In order to extract hot-water soluble proteins, more water is added, followed by heating. Protein extraction rate depends on the amount of water added. Optimal amount of water is about 10 times soybean weight. Insufficient amount of water will lower the extraction rate; for example, 80% of total protein can be extracted from soybeans and water in a 1:10 ratio, and 35% of total protein extracted from soybeans and water in a 1:5 ratio. A soybean slurry of 11 kg should be recovered from 1 kg of soybeans.

The silicone antifoaming agent can be added while heating to 100°C for 5–10 min. Direct or indirect steam can be applied for the thermal treatment. Heating temperatures lower than 90°C will produce soymilk with beany flavor, poor coagulation, and low extractable solids. Temperatures exceeding 100°C will not increase the extraction rate. Properly heated soybean slurry should be filtered through cheesecloths to obtain the soymilk.

b. Making of Tofu When the temperature of soymilk drops to 70–75°C, warm suspension of calcium sulfate (2.5 to 2.7% by the weight of raw total soybean) is added with vigorous stirring with a wooden spatula. After stirring, the mixture is let set for 10 min to coagulate the soy protein. In Japan, 2% of coagulant is used with less stirring to form bigger coagulated curds for the making of tofu. The making of sufu requires vigorous stirring to form small soy protein curds, which are easier to drain during pressing. Lowering the amount of moisture in the tofu will produce a harder-texture product. Either acidification of the soymilk to pH 5.0 by the addition of acetic acid along with coagulant or addition of 50 ppm antibacterial agent to the soymilk can prevent the unwanted microorganisms in curd molding.

2. Preparation of Moldy Tofu

This procedure is unique in sufu processing. The press-dehydrated tofu is cut into 2 cm × 2 cm × 2 cm cubes. If necessary, tofu cubes can be sun-dried or heat-dried to further remove surface moisture. The dried cubes are laid out in a rice straw–layered container, 2 to 3 cm apart from each other. Several covered containers can be piled up and placed in a warm environment. Fungi from the rice straw will be naturally transferred to the tofu cubes and grow. In 3 to 7 days, mold growth is complete, with white *Mucor* mycelia covering the surface of tofu cubes (Fig. 2). Homemade sufu uses natural fermentation to produce the moldy tofu. In industrial practice, pure culture is applied onto the surface of sterilized filter papers or bamboo sheets. They are layered with the tofu cubes for inoculation and fermentation in the incubator.

Processing time required for the preparation of moldy tofu depends on the fungi strains and fermentation temperature. *Rhizopus chinensis* var. *chungyuen* requires 7 days at 12°C, whereas *Mucor hiemalis* and *Mucor silvaticus* take only 3 days at 20°C, *Mucor praini* takes 2 days at 25°C, and *Actinomucor repens* needs 2 days at 27°C.

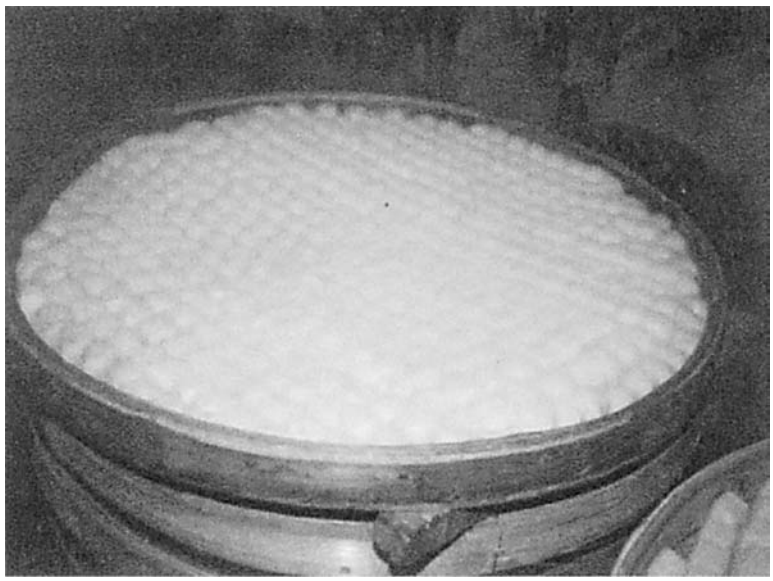

Figure 2 Molded tofu.

3. Brining and Maturation

Moldy tofu cubes can be sun-dried to remove the surface moisture followed by brine curing and mash aging. There are two methods for brine curing. One is to soak the moldy tofu cubes in saturated brine that contains 25–30% of salt to help the sodium chloride penetrate into the tofu interior. The other one is to spread salt on top of the moldy tofu cubes directly, followed by applying pressure to remove the moisture and effectively dehydrate the cubes. If moldy tofu is cured in high-alcohol-content mash or high-salt content mash, the brine-curing procedure can be eliminated (Fig. 3). There are some products called moldy tofu or salty sufu made only by brine curing and aging for more than 1 month.

After brine curing and partial dehydration, the tofu cubes are soaked in the mash for the aging process. Soaking in different mash results in different flavors and textures. Various mash formulations developed in different regions show their unique taste and flavor. Three categories of mash have been developed: (a) wine mash produced from grains; (b) soy sauce mash or miso, which is made from wheat and soybean; and (c) formulated mash containing alcohol and seasonings. There are many varieties of sufu manufactured with wine mashes. Sufu made with Shao-Shin liquor mash mixed with black bean koji, seasoning, and salt is highly rated. The salt-cured, moldy tofu cubes and olive leaves are layered in big ceramic jars. The jars are then filled with the mash, sealed, and aged for months or 1–2 years. Some manufacturers inoculate *Monascus purpureus* or *Monascus anka* on steamed rice to have red koji mash or Japanese-style cloudy wine mash. The product obtained from red koji mash is called red sufu. The mash in (b) that uses soybean koji or wheat koji blended with high concentration of salt and water for preservation is very salty. The formulated mash in (c) is a modified method for mass production. There are many formulations for the mash; for example, 10% alcohol blend with 5% salt or with 4% salt and 1% acetic acid. Either one of the formulations is good for preserving the sufu

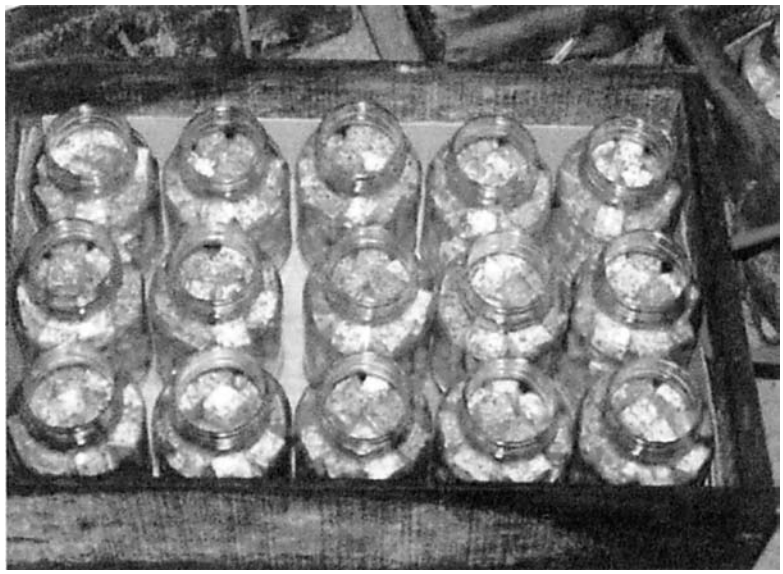

Figure 3 Bottling of seasoning tofu.

at 20–35°C for over 6 months. After packaging, addition of a layer of sesame oil or paraffin (food grade, MP 60°C) at the top of the jars can prolong shelf life.

C. Changes in Sufu During Fermentation

Sufu is an easily digestible soy food made from hot water–extractable soy proteins in the form of tofu that has passed through the fungal degradation, brine-curing, and mash-aging processes. The compositional changes are the result of salted migrating into the tofu cubes. When moldy tofu is converted to sufu, salt and the mash components migrate into the tofu cubes, whereas the soluble materials in the tofu cubes are transferred to the soaking liquid. Therefore, the characteristics of the aging mash will affect the composition of sufu during aging. Protein losses are 5% from tofu to moldy tofu, and 20% from moldy tofu to sufu, respectively (on dried weight basis).

Protein degradation occurs during sufu fermentation. In moldy tofu, protein nitrogen decreases significantly, and the amino nitrogen and ammonia nitrogen increase accordingly. Fungi alone cannot produce of ammonia nitrogen more than 7%, and thus bacteria may have contributed to the increase in ammonia nitrogen. In the final product sufu, protein nitrogen increase results from the soluble solids moved from the tofu cubes to the liquid and changes the overall percentages.

During sufu fermentation, a significant amount of cystine is lost due to degradation by microorganisms. None or trace amount of cystine can be found in sufu after a long period of aging.

D. Product Quality and Compositions

Sufu contains high levels of salt and spices. The nutritional composition of sufu variants sold in Taiwan is listed in Table 1. Sufu contains 60–70% moisture and 12–17% protein,

Table 1 Compositions of Sufu Variants Marketed in Taiwan

Composition	Rose sufu	Tsao sufu	Red sufu	Kwantung sufu[a]
Moisture	60.0	69.0	61.0	74.0
Protein	17.0	13.0	15.0	12.0
Lipid	14.0	13.0	14.0	6.0
Fiber	0.1	0.1	0.4	0.1
Sugar	0	0	0	0
Ash	9.0	5.0	9.0	7.0
Total N	3.0	2.0	2.4	2.0
Protein N	2.0	1.0	1.6	1.0
Non-Protein N	1.0	0.8	0.8	0.7
NH_3 N	0.2	0.2	0.2	0.2
Amino N	0.3	0.3	0.3	0.2
Other N	0.3	0.3	0.3	0.3

[a] Units by percent.
Source: Ref. 2.

including 63–68% protein nitrogen, 10–12% amino nitrogen, and 7–10% ammonia nitrogen. The ash is contributed from salt. Compared to miso and natto, the level of protein disintegration of sufu is low, but further breakdown of protein molecules can result in a flavor alteration. Rose sufu is the one aged with rose essence. Tsao sufu is aged in the wine mash added with clove and orange essences. Red sufu is aged in *Monascus* koji and soy sauce mash. Kwantung sufu is aged in salt, *Monascus* koji, white pepper, and anise.

E. Packaging

The critical step in sufu processing is the surface dryness of tofu cubes before fermentation. Covering the tofu cubes with a piece of cloth during sun-drying avoids dust and insect contamination. In common practice, products are packed in wide-mouth, glass jars or in cans. The important step in packaging is that sufu is layered in the jars. The jars are then filled with the remaining liquid and sealed tightly for preservation. Sufu that possess unique aroma and a nice cubical shape and is without surface contamination is recognized as a good-quality product.

F. Sanitary Sufu (3)

A modified processing technique was developed by Academia Sinica (Taipei) to produce sufu under more controlled sanitary condition with resultant good eating quality and storage property. Pure mold was cultured on filter paper and used to cover the sterilized tofu cubes. The moldy tofu stocks are ready after incubation at 12°C for 4 days. The tofu cubes are brined in 6% NaCl for a month, then dried to remove the excess moisture at 60°C. Such a product had stronger flavor and harder texture than the traditional product. This sanitary sufu could be spread on bread and is stable for storage as cheese. Food-grade paraffin was melted and used as sufu wrapper that could be peeled off before consumption. Additional plastic film wrapping could also be used before paraffin wrapping. Another packaging method is to use CO_2 gas to replace air for canning purpose.

III. CHAOTOFU OR STINKY TOFU

A. Introduction

Chaotofu or stinky tofu is one of the traditional Chinese soy foods. It is still made by the traditional, household, open-fermentation method. The tofu is soaked in special stinky brines for 4–6 hr, to conduct the brief fermentation. The stinky brine is made by letting the various ingredients in the brine carry out a natural fermentation with production of a strong stinky odor. These ingredients can be (a) various vegetables such as amaranth leaves, bamboo shoots, and winter (wax) melon, (b) salted mustard brine with shrimp and salted egg brine, (c) fish, shrimp, and animal organs as the main ingredients, or (d) a strong alkali such as ammonia (for rapid method).

The principle behind for making of stinky tofu is the utilization of proteolytic enzymes from microorganisms in the stinky brine to partially hydrolyze the proteins in tofu to make the soy proteins more easily digestable. Because it is a still, anaerobic fermentation process, odorous proteolytic intermediate metabolites such as ammonia are produced in the brine, and thus the brine is called stinky brine. These odorous metabolites adhered on the surface of the tofu are vaporized during the deep-frying or steaming process. At present, no pure culture for making stinky tofu is available commercially.

Stinky tofu has to be cooked, either by deep-frying or steaming, before consumption. Seasonings include chili sauce, garlic sauce, and/or soy paste.

B. Making of Stinky Tofu

1. Raw Materials

a. Tofu Making Tofu is made by following the procedures described previously in the sufu section. However, a so-called hard tofu with less moisture content is preferred. The recovery rate for hard tofu is only 60–75% of regular tofu. Tofu with moisture content less of than 75%, such as dry tofu, is not that suitable, because the final product is too coarse.

b. Stinky Brine Preparation The source of stinky brine starter can be the original stinky brine or mashed raw stinky tofu. Either added to the stinky brine media followed by fermentation for 1 week. Another source is mixed culture isolated from stinky tofu containing *Bacillus* sp., *Streptococcus* sp., *Enterococcus* sp., and *Lactobacillus* sp. The mixed culture is inoculated on trypticase soy agar followed by 24 hr incubation at 30°C. All the colonies are then transferred into sterilized culture media followed by incubation at 30°C for 30 days or longer to make the stinky brine starter. The stinky brine media can be made by using formulations such as: (a) cabbage (30%, w/v), bamboo shoot (20 %, w/v), tofu (30%, w/v) shrimp (5%, w/v), and salt (1%, w/v); (b) cabbage (30%, w/v), bamboo shoot (20%, w/v), tofu (40%, w/v), and salt (1%, w/v); (c) a mixture of the above two formulations. The formulations are mashed and sterilized at 121°C for 45–60 min. The media is inoculated with 5% stinky brine starter, followed by still fermentation at 30°C for 1 month or longer.

A household method of preparing the stinky tofu brine medium is as follows. Use amaranth leaves and mature ginger slices (1:2), or amaranth leaves, mature ginger slices, and salted mustard (1:2:1) as the raw ingredients. The whey collected from making tofu is used to cover the above ingredients, with or without addition of 0.5% original stinky tofu brine. Ferment for 2 months (summer) to 6 months (winter) until the ginger slices can be smashed easily with fingers. Addition of too much salted leaf mustard will make the brine

too salty. Addition of bamboo shoot is also practiced, resulting in less turbid brine. Addition of 5% fish meat to the basic formulation is another option. Ammonia is added in the so-called rapid fermentation method. Use of tofu in the formulation will make the brine viscous. The final product has to be washed before deep-frying. The alternative is to use tofu whey. Another procedure for making the stinky tofu brine is to mash the raw stinky tofu and add it to the stinky tofu media, followed by 1 week of fermentation.

2. The Stinky Tofu Making Process

A flowchart for making stinky tofu is presented in Fig. 4. Tofu slices (4.5 cm × 4.5 cm × 1.2 cm) are put into a plastic container. Enough stinky tofu brine is added to cover them. They are held at room temperature for 4–6 hr, or at 5°C for 20 hr or longer (Fig.5). The holding period is longer in newly prepared stinky tofu brine, as compared to the shorter holding period in used brine. Holding too long will breakdown the tofu or make the odor too strong. The fermented tofu is then drip-dried at 4°C overnight, at the same time shrinking the tofu (Fig. 6). The stinky tofu is ready for consumption after deep-frying at 150–160°C or steaming.

3. Changes During Fermentation

Changes in tofu during the making of stinky tofu have been reported (4). When *Bacillus sphaericus* is cultured in the brine medium containing meat and fish, the ammonia content in the brine increased from about 100 mg/L to about 3400 mg/L in 10 weeks. During the same period, the pH value dropped from 6.5 to about 4.7 in the first week and then

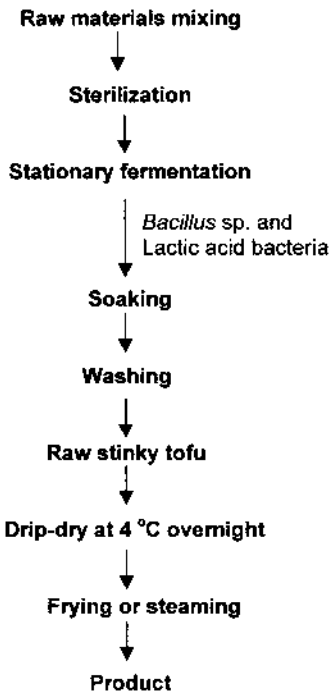

Figure 4 Flowchart for making stinky tofu. (From Ref. 4.)

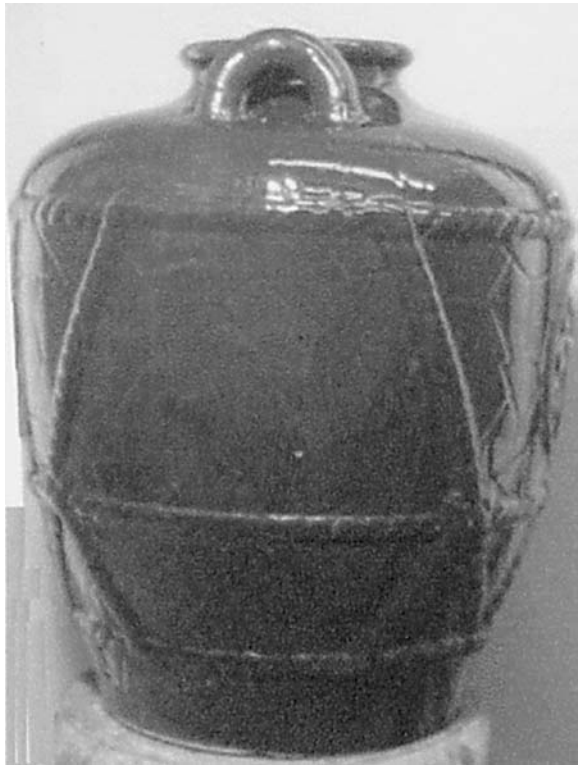

Figure 5 Dipping of stinky tofu.

Figure 6 Dipped stinky tofu.

increased gradually to about 7.5. The total bacterial count fluctuated between a light decrease after 10 weeks of fermentation.

In the first week of the fermentation period, the pH value of tofu slices in the stinky tofu brine dropped from 6.5 to about 4.6 due to the production of lactic acid and growth of the lactic acid bacteria. Thereafter, the protein in the tofu was hydrolyzed by the microbial proteases to form amino acids, followed by the action of deaminase to form ammonia. The pH value therefore increased gradually to about 7.5 in week 10. Ammonia content continues to increase, favoring the growth of more alkali-tolerant bacteria instead of the lactic acid bacteria, with a decrease in total bacterial count (4). Because of this phenomenon, stinky tofu is considered an alkaline, fermented food (5).

It is understood that types of bacteria for various formulations will differ. From this particular fermentation, *Bacillus* sp. such as *B. megaterium*, *B. polymyxa*, *B. pumilus*, *B. subtilis*, and *B. sphaericus*, and lactic acid bacteria such as *Enterococcus*, *Streptococcus*, and *Lactobacillus* sp. were isolated from the brine and from the tofu surface. At the beginning of the fermentation, the lactic acid bacteria such as *Enterococcus*, *Streptococcus* and *Lactobacillus* prevailed. As fermentation progressed, the ammonia resulting from the hydrolysis of protein caused the pH value to increase. Therefore, at the later stage of fermentation, the more alkali-tolerant *Bacillus* sp. dominated. It was found that quality of stinky tofu is better from mixed cultures as compared to single cultures (6).

4. Product Quality

In the traditional method of making stinky tofu brine, it is common to use salted leaf mustard brine or rotted, salted leaf mustard, shrimp, and brine for making thousand year old egg or pedan (brine composed of salt, tea leaves, wine, and potash). Fermentation relies on the microorganisms from these ingredients. Therefore, differences will occur from different batches of raw materials with inconsistent quality. In addition, the use of large chunks of raw materials makes digestion by microorganisms a very slow process, up to 6 months. During this period, because of the use of an open system, the brine is vulnerable to insect infestation. In the stinky tofu brine made by the household method, the pH value is between 5 and 7, with 1–3% salt, and has a total bacterial count of 10^7–10^9 cfu/mL. Quality is not consistent. With the use of pure cultured stinky tofu starter to make the brine, the making of stinky tofu is sanitary and safe, with consistent good quality.

Deep-fried stinky tofu has the following nutrient composition (per 100 g): total solids, 30.37 g; protein, 15.22 g; fat, 11.94 g; ash, 1.07 g; vitamin B_1, 0.048 mg; vitamin B_2, 0.03 mg; niacin, 0.43 mg; iron, 2.9 mg; potassium, 94 mg; sodium, 58 mg; calcium, 131 mg; magnesium, 47 mg; and phosphorus, 220 mg (7).

When mixing new and used brine, the soaking period of tofu in the brine has to be adjusted accordingly. Soaking too long will cause easy damage to the integrity of the final product, and too short a soaking period will result in a low quality product. Temperature of deep-frying should not be too high; it should be maintained at medium heat. The center of the tofu pieces must be fully cooked with the surface golden yellow. If not, diarrhea may occur.

5. Sanitation Control

In the manufacturing of stinky tofu, the traditional procedure for preparing the stinky brine is to use raw salted vegetables. These vegetables carry the microorganisms that come with them, and thus the process is a natural fermentation. Consequently, the quality is not stable and varies among batches. In addition, this lengthy, open fermentation method to

induce fermentative microbial growth attracts flies and insects, which lay eggs that can change into maggots. Pathogenic microorganisms can also grow and are harmful to the consumers. It is therefore important that in the preparation of stinky brine, the medium should be sterilized and followed by inoculation with pure cultures of *Bacillus* sp. and other lactic acid bacteria to prepare sanitary brine for making stinky tofu. The tofu blocks used should also be as fresh as possible. After soaking in the stinky brine, they should be washed with clean water and left at 4°C for drip-drying. The pretreated tofu can then be deep-dried to become sanitary stinky tofu.

Stinky tofu belongs to the alkaline fermented food category. The amino acids in them will not be decarboxylated and form amines. Amino acids will form amines through decarboxylation only under acidic condition. In neutral and alkaline conditions, the deamination process will produce ammonia. Therefore, stinky tofu has a strong ammonia odor. During deep-frying, the ammonia volatilized does not post a health problem.

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32
Tempeh: The "Other" White Beancake

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I. INTRODUCTION

Most Americans in the year 2000 could identify tofu as soybean cake originating in China and Japan, a very small percentage had some knowledge of tempeh, the fermented soybean or grain cake from Indonesia (Fig. 1). For the purpose of this chapter, tempeh (or tempe, pronounced "tem-pay") is defined as a fermented cake of soybeans or grains that has been a traditional staple of the Indonesian people for centuries.

In the year 2002 in North America, tempeh production was about 14,000 lb (ca. 6364 kg) per day, with three major manufacturers producing about 90% of the volume. The market value of this volume is about $10 million per year. The average weekly consumption per person in Denpasar (Bali, Indonesia) and Yogyakarta (Java, Indonesia) is 75 g and 200 g, respectively. A large manufacturer produces about 5000 pounds of tempeh a day in Yogyakarta, Indonesia, but with a market value of less than $1000. However, when adjusted to the per capita income in these two countries, a cake of tempeh is about 4 times "cheaper" in the United States. In Indonesia, tempeh was considered the poor person's food in the past, but now it is proudly viewed more as a precious part of the unique cultural heritage of Indonesian cuisine (1).

II. THE HISTORY OF TEMPEH IN INDONESIA AND THE UNITED STATES

Tempeh is unique among soyfoods in that it did not originate in China or Japan. It originated on the Island of Java in Indonesia prior to 1800, and maybe as long ago as a thousand years. Not much is known about the origins of this food except that it was part of a group of fermented foods that were developed by people with no scientific background in food sciences or fermentation. Tempeh was made not only on soybean substrates but on a variety of agricultural by-products such as peanut and coconut presscake and okara, or soy pulp, which is left over after the production of tofu.

Although, the earliest references in Indonesian literature were only in the early 1800s, tempeh is believed to have been developed long before that time due to the widespread geographical distribution of the product throughout Java and all of Indonesia. Tempeh is a major part of the cuisine of Indonesia where it is made by more than 40,000 cottage industry sized shops using very basic tools and open-air fermentation (2).

Figure 1 Example of tempeh sold at an Indonesian market.

One argument has been advanced that tempeh originated some 2000 years ago, brought to Indonesia by Chinese traders who were at that time already fermenting soybean koji for their soy sauce. This technique could have been modified by local Indonesians to suit Javanese tastes and climate—*Rhizopus* is better adapted to the heat and humidity of the tropics than the *Aspergillus oryzae* used by the Chinese in their koji (2).

The first Westerners to study tempeh were two Dutch scientists, H.C. Prinsen Geerligs and F. A. Went, who were studying the utilization of sugar by-products from the Dutch sugar plantations in the late 1800s. Indonesia was a Dutch colony from the late 1600s through the 1960s, hence much of the early scientific work was done by the Dutch (2).

During World War II, a Dutch microbiologist, Van Veen, was held as a POW (prisoner of war) along with many American soldiers by the Japanese. Van Veen noted during postwar studies that tempeh was much easier to digest than plain cooked soybeans. He concluded that many POWs, especially those with dysentery and edema, owed their lives to tempeh, which gave them badly needed protein that they could easily assimilate (2).

The first American researcher to study tempeh was Dr. Clifford Hesseltine of the Northern Regional Research Center in Peoria, Illinois. There, Dr. Hesseltine concluded that the primary microorganism used to ferment tempeh was *Rhizopus oligosporus* and not *Rhizophus oryzae* as previously thought. He also developed a method of fermenting the tempeh in perforated plastic bags instead of the commonly used banana leaves. This bag method quickly became the most commonly used method of incubation in Indonesia and later on in North America as well (2).

In the 1970s, tempeh was first brought to the attention of the American public largely through the efforts of The Farm, a spiritual community of 1700 "hippies" in southern Tennessee. These people were total vegetarians (vegans) and lived off soybeans. In an attempt to find new ways of preparing soybeans, Alexander Lyons learned about tempeh after doing some research in the NIH libraries in Washington, DC. Alex Lyons, along with Cynthia Bates, developed a home tempeh starter kit with instructions and set up a small lab at The Farm to produce the necessary spores. This is where most of the tempeh pioneers—such as Seth Tibbott of Turtle Island Foods, Michael Cohen of Lightlife Foods, Steve

Demos of White Wave, and Jerimiah Ridenhour of Wildwood Natural Foods—learned about tempeh and its manufacturing techniques.

By the 1980s, tempeh was being touted as the new wonder food throughout North America, Europe, and parts of Asia, most notably Japan. Several producers of a Japanese fermented soybean product, natto, banded together to study tempeh and produce a breaded tempeh filet that consumers fried at home in woks. Immediately they became the biggest tempeh producers in the world, only to fold their operations a few years later.

In the United States, tempeh got a big boost from the production of the first Tempeh Burgers by Pacific Foods in San Francisco. Relatively few vegetarian burgers existed at that time, and these became quite popular. Pacific Foods also produced the first vacuum-sealed tempeh, a method that soon became the predominant method of packaging tempeh in North America. Multigrain tempehs were first introduced when Turtle Island Foods produced its Five Grain Tempeh in 1980 (Fig. 2).

Figure 2 Multigrain tempeh sold in the United States.

As the 1980s wore on, tofu, not tempeh, became America's favorite soy food of choice due to the lack of an ethnic base for tempeh and a lack of knowledge on how to prepare the product. Also, products such as the Gardenburger, which were easier to prepare and cheaper to manufacture, began to capture the public's attention. Tempeh manufacturers began to consolidate, and some stopped making the product altogether. Currently the North American tempeh market is dominated by three key players—Lightlife of Massachusetts, White Wave of Colorado, and Turtle Island Foods of Oregon—who collectively controlled 90% of the market for tempeh in North America in 2002.

In Indonesia, tempeh is still widely consumed by all economic classes of society. Once perceived as only a poor person's food ("Don't be a Tempeh Nation," General Sukarno exhorted in the 1960s), tempeh today is proudly viewed more as a precious part of the unique cultural heritage of Indonesian cuisine. It is still made in small shops on a cottage-industry scale with virtually no large, modern plants. As such, tempeh plays an important role in the economy and fabric of life of the Indonesian people (1).

III. BASIC CHANGES IN THE TEMPEH FERMENTATION PROCESS

What happens during the fermentation of soybeans into tempeh? Generally it is agreed that not only is the flavor, aroma, and texture of plain cooked soybeans enhanced during the fermentation process, but there are several nutritional benefits derived in the process as well to make the beans more easily assimilated by the human body. This is done by the main mold *Rhizopus oligosporus,* and other minor organisms or their enzymes hydrolizing proteins, carbohydrates, and fats to create smaller and more digestible units. This process makes the protein in tempeh more digestible and usable.

A. Changes in Lipids

Free fatty acids increased from 0.5% in the unfermented control to 21.0% in the dehydrated tempeh (with the same moisture content). During fatty acid synthesis, *Rhizopus* sp. produced only gamma-linolenic acid (GLA) instead of alpha-linoleic acid. GLA is a prostaglandin and leukotriene precursor. It is used therapeutically to decrease the cholesterol and triglyceride content in blood. It is not found in soybean (3).

B. Changes in Carbohydrates

During fermentation, the principal changes in carbohydrates are the rapid decrease of hexoses and the slow hydrolysis of stachyose, the flatulence factor in beans (4). This makes tempeh a more socially acceptable soybean product.

C. Changes in Proteins and Amino Acids

Steinkraus (5) summarized the biochemical analysis of tempeh. The most significant changes are in the proteins and vitamins. Ammonia (% of total nitrogen) increased from 0.1 to 1.7. Percent nitrogen soluble in water increased from 6.5 to 39.0. Percent nitrogen soluble in trichloroacetic acid increased from 5.9 to 28. There were no significant changes in the amino acid patterns between soybeans and tempeh (6). It is likely that there is no de

novo synthesis of amino acids, but only a degradation and consumption of soy protein by the fungi.

D. Changes in Vitamins

Steinkraus (5) also summarized reported work on changes in vitamins during tempeh fermentation. Riboflavin increased by 2–47 times, niacin increased by 2–7 times, and vitamin B_{12} by 33 times. Thiamin, unfortunately, decreased. Panthothenic acid has been reported to stay the same or increased by 2–4 times. Pyridoxine increased by 4–14 times. Biotin and total folate compounds were respectively 2.3 and 4–5 times higher in tempeh than in unfermented soybeans. The variations in reported changes may be due to the way tempeh was made in various laboratories and locations, as well as the beans and microorganisms associated with them. For example, according to this author's experience, the production of vitamin B_{12} fluctuated considerably even under similar fermentation conditions in the same location.

E. Presence of Antioxidants and Antibiotic in Tempeh

An isoflavone identified as 6,7,4′-trihydroxy isoflavone (called Factor 2) has been reported (6,7). The antioxidative effect of factor 2 on retinol was about the same as DL-alpha-tocopherol, and three times that of genistein. Other isoflavones were later reported (8,9).

In Indonesia, tempeh is widely used to ween babies off mother's milk and to help patients recover strength from dysentery and other ailments of the intestinal tract (1). *R. oligosporus* NRRL 2710 was reported to produce an antibiotic active compound against a number of gram-positive bacteria including *S. aureus* and *B. subtilis,* as well as the gram-negative *K. pneumoniae* (10). It was demonstrated later that *K. pneumoniae* and *R. oligosporus* NRRL 2710 grows well together in tempeh fermentation. There was no evidence of *K. pneumoniae* inhibition by the mold (11,12). This may help explain why tempeh is provided to patients with dysentery and other ailments of the intestinal tract.

F. Reduction of Phytate

Phytate is considered to exacerbate mineral deficiency in human by hindering absorption in the gut. Reduction of phytate was reported to be 22% during tempeh fermentation due to phytase active in *R. oligosporus* (13). This again demonstrated the benefits of tempeh.

IV. TEMPEH FERMENTATION IN INDONESIA (1)

A. A Small Tempeh Manufacturer in Denpassar, Bali

A home-based tempeh factory can be operated by a small family group of four adults and several small children. Fifty kilos of tempeh are made each day in the house. Cleaned soybean is cooked in the early morning. At mid-morning, the cooked beans are cooled manually in a large bamboo colander placed on the floor. The tempeh from the day before is incubated in small 3" × 3" perforated plastic bags, each of which weigh about 3 ounces. These small cakes are incubated for 2 days on wooden slats in a dark room. A bicycle-powered mill, the colander, and an aluminum cooking pot and heat sealer are the only pieces of equipment. The 3-ounce cakes are sold to restaurants and in the market for 150 rupiah (about 6 US cents).

B. A Medium-Scale Manufacturer in Denpassar, Bali

A substantial medium operation produces 750 kilos of tempeh, 7 days a week, 30 days a month. The manufacturer employs 10 young men in this operation.

The soybeans are cooked in delidded 55-gallon drums placed over propane burners. The hulls are skimmed off the beans manually with plastic colanders by two persons. Two others cool and package cooked beans in perforated plastic bags in another room. The beans are piled not on the floor but on a piece of white canvas. Tempe Murni sells 250 grams of finished tempeh for 400 rupiah (U.S. 16¢) to their distributors, who sell it in the market for 500 rupiah (U.S. 21¢).

C. A Large Tempeh Manufacturer in Yogyakarta, Java

Yogyakarta has always been viewed as the cultural capital of Indonesia, it has the highest per capita consumption of tempeh in all of Indonesia. On a weekly basis, the average person in Yogyakarta consumes nearly 200 grams of tempeh versus only 75 grams per Balinese citizen. This shows in the local market which is totally inundated by a large variety of tempeh products—both raw cakes and tempeh prepared in various sauces. These products are supplied mainly by one large tempeh maker. This innovative plant produces 5000 pounds of product each day from a 1500 square foot area. This shop does have a gas-fired boiler and copper kettle for cooking the beans but everything else is done with the same level of technology witnessed elsewhere (bamboo colanders on the floor for mixing and packaging, etc.). What is unique about this shop is the ingenious barter system that exists here. The main operation is run by a paid staff of 24 people but the filling is almost all done manually by local workers, They seal the inoculated beans in plastic bags, sometimes melting the plastic by running it near the open flame of a cloth wick stuck into a coke can filled with kerosene! After the bags are sealed, the workers pack them up into the cloth sacks the beans came in, load them onto bicycles, and pedal the load home. There they incubate the beans for several days, and when the beans are white and ripe, take them to the local market for sale. In Yogyakarta, small, 1 ounce packages of tempeh incubated for an extra long time in banana leaves are available and sold for 25 rupiah (about U.S. 1¢).

In Indonesia, the same basic processing steps for tempeh making takes place. Figure 3 presents a generalized flow chart on these procedures followed in Indonesia (1,14). The steps are as follows:

1. Beans are soaked overnight in what is known as the prefermentation.
2. Early the next morning the beans are dehulled and split, using anything from mills to hands and feet.
3. Beans are cooked in open kettles where more dehulling takes place.
4. Cooked beans are placed in a large woven colander about 3 feet across and placed in front of fans to dry.
5. Cooled beans are inoculated with the culture. Most culture comes from the same source, a local Indonesian lab, and is a type of Rhizopus that is incubated at around 30°C for 48 hr.
6. Inoculated beans are now scooped into perforated plastic bags or banana leaves. Whereas the perforated plastic bags were the most common way of incubating tempeh, most cooks preferred tempeh incubated in banana leaves. Observations in the market bore this out as tempeh incubated in the leaves was consistently of higher quality than that in the plastic bags.

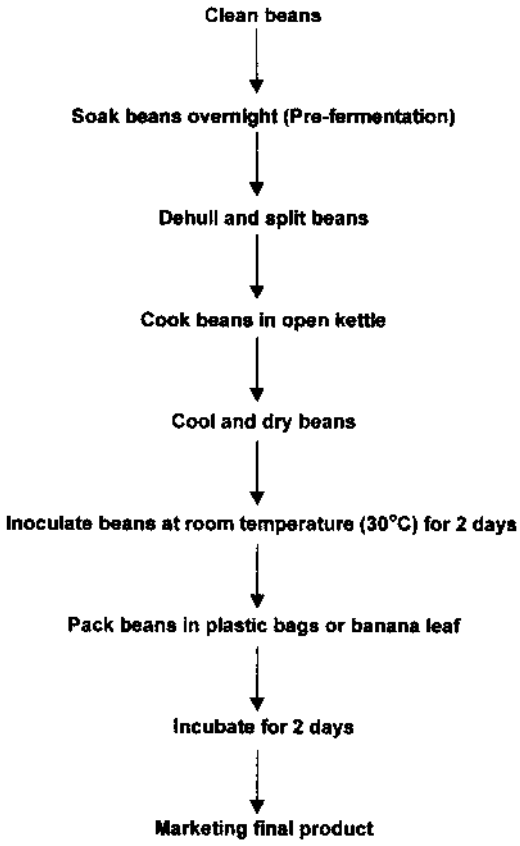

Figure 3 Flowchart of tempeh manufacturing in Indonesia.

7. Incubation takes place in a room or closet where inoculated beans sit out at room temperature on wooden slats.
8. After 2 days the tempeh is sold in its perforated incubation bag or banana leaf in the marketplace.

V. TEMPEH MAKING IN NORTH AMERICA, CA 2000

In the year 2000, only about seven shops existed in North America capable of making a thousand pounds of tempeh or more per day. These were Lightlife Foods in Massachusetts, Turtle Island Foods in Oregon, White Wave in Colorado, Northern Soy in New York, Cricklewood Foods in Pennsylvania, Surata Soyfoods in Oregon, and 21st Century Foods in Massachusetts. Even the smallest of these would be viewed as a sanitary modern factory compared to Indonesian methods. Most shops follow a flow chart or processing scheme (Fig. 4) similar to the following:

1. Whole soybeans are heated and split dry in a mill.
2. The split beans and hulls fall into a tube where the lighter hulls are sucked out from the top by connecting it to an exhaust fan. The heavier beans fall to the bottom of the tube into a collection bucket.

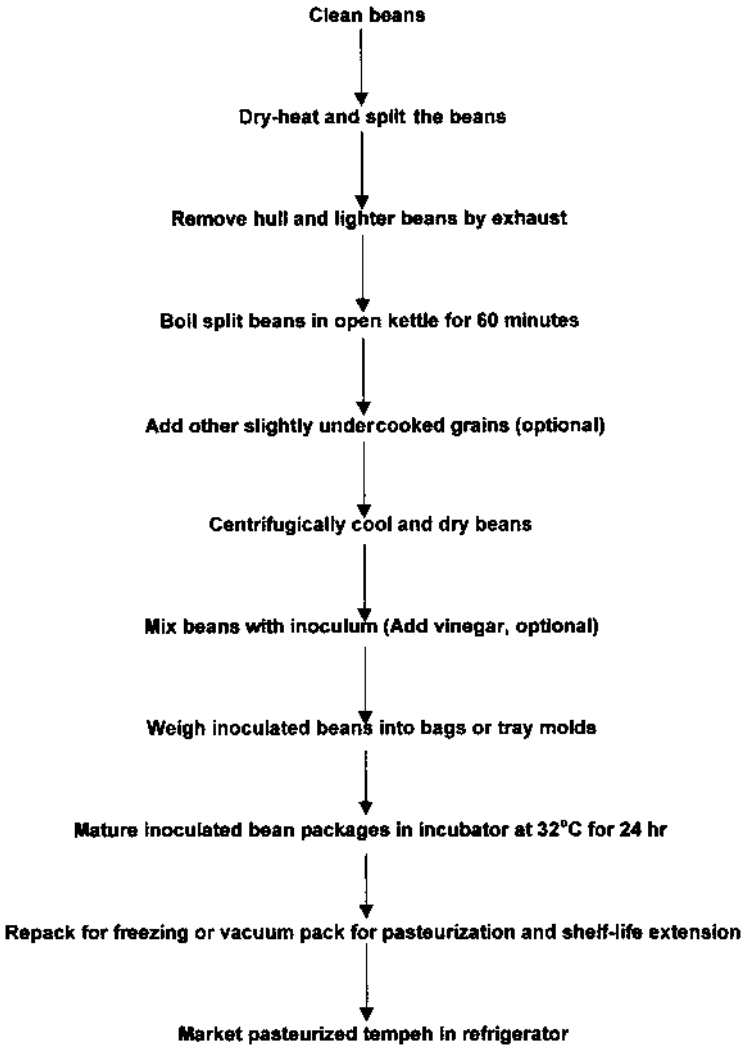

Figure 4 Flowchart for tempeh manufacturing in the United States.

3. The split beans are cooked at a boil for 60 min in an open steam jacketed stainless steel kettle.
4. Other grains may be added toward the end of the cooking time. These grains are always slightly undercooked. The rule here is to cook the grains for about half as long as one normally would if one were preparing them to eat at our own supper table.
5. Cooked beans are now placed into a centrifuge. These have stainless steel baskets and the beans are spun rapidly for a short period of time to cool and remove excess water.
6. Cooled beans are placed in a horizontal mixer where they are mixed with the innoculant and other grains. Some people acidify the beans with a small amount of vinegar at this point.

7. Beans are now either placed in a mechanical scale of some kind or weighed out by hand into perforated bags or tray molds for burgers.
8. Bags of inoculated beans are now laid flat on perforated trays and placed inside an incubation room where they are heated at 32°C (89.6) for 24 hr.
9. At this point tempeh is now bound into a firm, fragrant white cake. Some workers slip an outer bag over the tempeh at this point and freeze the product.
10. Most commonly at this point, finished tempeh is now vacuum packaged and then steam or hot water–pasteurized to extend the shelf life.
11. Tempeh is now cooled down and sold refrigerated it has a shelf life of approximately 3 months.

VI. MISCELLANEOUS ISSUES AND PROBLEMS OF MODERN-DAY TEMPEH PRODUCTION AND INCUBATION

The incubation of tempeh is a delicate balance of temperature, airflow, hole size on bags or trays, and relative humidity. Some common problems observed are as follows:

1. Hole size in incubation bags. This is an important feature and somewhat varies based on the incubator that the product is placed in. If the airflow, temperature, and humidity are all adequately controlled, it is possible to get away with a larger perforation than in an incubator with inadequate controls. As a rule, 1.5 mil polyethylene bags with size 7 sewing needle perforations every one-half inch will produce a quality product.

2. Incubation. During the first 12 hours, the inoculated beans are just heating up and no cooling is required, assuming the temperature of the incubator is around 88°F (32°C). After 12 hr, a small amount of water begins to collect on the inside of the bags. At 14 hours, fermentation of the *Rhizopus* spores has begun in earnest and active cooling of the air must take place. If the inoculated beans reach a temperature above 92°F (33.3°C), conditions are no longer ideal for *Rhizopus* spores but are ripe for a different set of organisms such as those of the Bacillus group. *Rhizopus* can be severely damaged by heat of over 92°F (33.3°C).

At temperatures above 96°F (35.6°C), *Rhizopus* is killed and the product will be discarded. If internal temperatures don't reach 96°F (35.6°C), the tempeh can usually be saved. Heat-damaged tempeh may be taken out of the incubation room and kept out at room temperature (approximately 70°F or 21.1°C), and it will start to grow over the damaged areas with new white mycelium.

The ideal relative humidity for incubating tempeh is between 50% and 75%. Airflow should be kept in the 120-cfm range to prevent overdrying of the product, which can lead to premature sporulation. One design for a tempeh incubation room that we have loosely followed was presented by Chananyah Kronenberg in the Summer 1983 issue of *Soyfoods Magazine*. The incubation room suggested in the article is presented in Fig. 5 (15).

3. Premature Sporulation of Tempeh. During incubation, sometimes black spots will show up around the holes of the bag. These are actually the spores of the tempeh culture and are not harmful to eat though they often lead to the consumer discarding the product as inferior. In some parts of Indonesia, black, overripe tempeh is sold in the market and used as a spice in much the same way a well-aged cheese may be used. But by and large, the black spots are the bane of the commercial tempeh-maker and should be avoided who ever possible. An albino form of *Rhizopus oligosporus* spores to be refined and sold commercially is highly desirable.

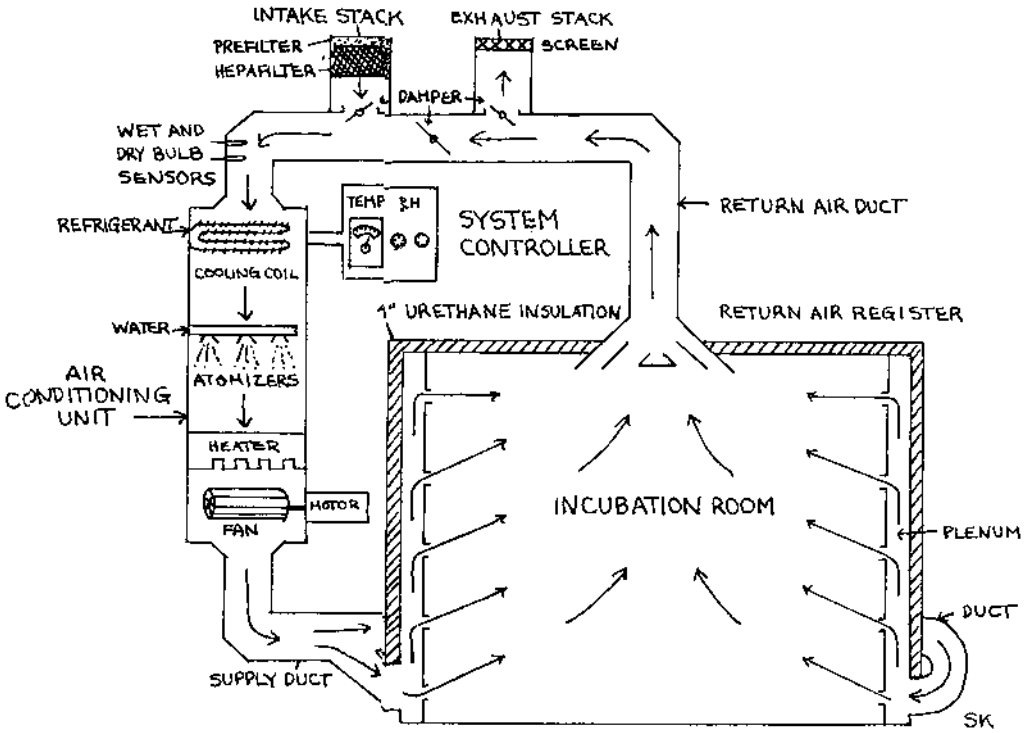

Figure 5 Integrated tempeh incubation system. (Drawing by Shira Kroneenberg. Permission from H. Jeff Kronenberg.)

Tempeh sporulates when a threat to the culture's existence arises. This can be from excessive drying due to too much airflow, heat, or lack of adequate humidity. When one of the above causes is to blame, the tempeh develops black spots around the perforations of the incubation bags. A pattern of black dots can be clearly seen on the wrapper.

Another kind of premature sporulation appears as a general blackness underlying the white mycelia on the product. This type of sporulation usually can be traced back to inadequate cooking times of the beans or the grains. If the beans or grains are undercooked too much, they will not fully hydrate and the product will blacken all over due to the inadequate moisture in all the beans.

4. Contamination. Whereas *Rhizopus oligosporus* is generally a very hardy mold that grows so strongly and quickly that other organisms are crowded out, there are three basic tempeh contaminants that can cause problems. The most severe contaminant facing tempeh makers are those from the *Bacillus* family. *Bacillus* contamination results from too much heat and too much moisture. It has a distinctive odor similar to a wet baby diaper. The cake will be dark brown and slimy to touch. Bacillus is a very virile bacteria and must be dealt with swiftly and thoroughly. Many tempeh makers have reported *bacillus* outbreaks in their plants that take weeks to resolve. The best move is to discard any *Bacillus*-contaminated cakes and immediately wash down floors, walls, and ceilings with a weak chlorine solution or quaternary ammonia. Bacillus problems seem to be most common in tempeh plants that share space with tofu-making operations.

Besides Bacillus, *Pseudamonas* (pink mold) and *Aspergillus* (green, "bread mold") are the only other microorganisms that will grow on tempeh in one tempeh processing plant. It

is a good manufacturing process to have tight control over the starter culture, which can also lead to contamination problems.

5. Tempeh Starter. Due to problems that can occur with contamination, the Indonesian practice of starting successive batches of tempeh from previous tempeh batches left to ripen in the incubator will post risks. Buying a good pure culture from a reputable lab and extending this on rice or other grains every month is a good manufacturing process. Basic instructions for producing your own tempeh starter can be found in the *Book of Tempeh* by William Shurtleff, Ten Speed Press, Berkeley, CA (16), and *The Complete Book of Tempe* published by the American Soybean Association (17).

6. Vacuum Packaging. About 85% of all tempeh sold in the United States today is sold vacuum-packaged. The first vacuum-packaged tempeh in the United States was introduced around 1980 by Pacific Soyfoods, a San Francisco company. At this writing, the four largest North American tempeh shops (Lightlife, Turtle Island Foods, White Wave, and Northern Soy) all vacuum-package their tempeh. After packaging, these tempeh cakes are pasteurized and sold with a 60- to 90-day refrigerated shelf life.

During vacuum-packaging of tempeh, several changes occur. First, and most obvious, is the disappearance of the white tempeh mycelium, which is surpressed into the substrate during vacuum-packaging. The result of this is a tempeh cake that appears brown and the individual beans are easily seen. Second, it is the belief of this author that the resulting tempeh often changes in flavor and texture, leaving the resulting product slightly more bitter and smoother in texture. The cause of this change in taste and texture needs to be studied further, especially the change in flavor.

The reason behind vacuum packaging is that the tempeh can now be sold refrigerated, alongside the tofu with a longer shelf life. Given the modern food distribution systems and enhanced movement of refrigerated products versus frozen products, it is hard to argue against the wisdom of vacuum-packaging tempeh. On the other hand, the changes brought on by this form of packaging create a different quality product, not necessarily for the better.

VII. CONCLUSION

Tempeh is a fermented food of Indonesia with a long, storied past and great potential yet uncertain future in the United States. Largely made on a small, cottage industry level where self-sufficiency is valued over financial gain, Indonesian tempeh is made today in thousands of shops all across this island nation using very rudimentary fermentation methods. Tempeh is an ethnic food that deserves the pride that Indonesians now place in this most unusual product.

Transferring this cottage industry into the modern Western realities of sanitary, large-scale food production brings many challenges and problems. From a production standpoint, perhaps the biggest problem is the fact that the incubation of tempeh takes up a large quantity of space and a relatively long period of time with high loss potential from fermentations gone awry. Other soy products made from soy protein isolates and concentrates are more suited to contemporary industrial food production because the processing of these foods takes up less time and space. Then there are the marketing challenges as one tries to educate the American public in what to do with a fermented cake of soybeans. Soy protein isolates and concentrates can be easily made with more and more accuracy, to simulate the more familiar tastes and textures of meat.

Still, as a whole food, made by a relatively simple technological process, tempeh has great potential. Tofu was brought to this country in the early 1900s by Japanese and Chinese immigrants and it was only in the 1980s that it became popular with the American public. And this popularity was driven somewhat from innovative soy products such as tofu dogs, burgers, and ice cream. Perhaps when secondary products are made from tempeh, such as fries, snacks and flavored frozen food entrées, tempeh will in time take hold in the Western world in much the same manner as tofu. The author's experience is that when prepared properly, tempeh is well loved and even preferred over many of the higher tech soy products.

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Fermentation: Principles and Microorganisms

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I. INTRODUCTION

The use of microorganisms to process or preserve foods is an ancient technique. Yeast was the first microorganism used in the production of wine and beer and the leavening of dough. These techniques have been known for at least 4,000–5,000 years. When these processes are underway, bubbles form as in gentle boiling. This bubbling is due to the liberation of carbon dioxide from the degradation of sugar. The word fermentation signifies the gentle bubbling or boiling condition in these processes.

The nature of the fermentation reaction did not become clearly understood until the late part of the nineteenth century when Louis Pasteur discovered the relationship between living cells and fermentation. In 1854, Pasteur demonstrated the relationship between yeast and this reaction. The word fermentation became associated with microorganisms. Pasteur also showed that true fermentation occurs only in the absence of free oxygen. He called life without air anaerobiosis. Actually, the definition of fermentation in biochemistry is the extraction of energy from carbohydrates and other organic substrates without using O_2 as an electron acceptor. Hence fermentation is an energy-yielding catabolic pathway that proceeds with no net change in the oxidation state of the products compared to that of the substrate. The common usage of the word fermentation frequently overlooks the strict biochemistry definition. A broad sense was adopted, that is, a process in which microorganisms produce chemical changes in organic substrates through the action of enzymes produced by these microorganisms. According to the common usage, the term fermented foods is used to describe a special class of foods that contain a complex mixture of carbohydrates, proteins, fats, etc., undergoing simultaneous modification under the action of a variety of microorganisms and enzymes. Reactions involving carbohydrates and carbohydratelike materials are referred to as fermentative. Changes in proteinaceous materials are designated proteolytic, and the breakdown of fatty substances are described as lipolytic. When complex foods are fermented under natural conditions, they invariably undergo different degrees of each type of change. Whether fermentative, proteolytic, or lipolytic end products dominate will depend upon the nature of the food, the types of microorganisms present, and the environmental conditions affecting their growth and metabolic patterns.

The basic concept of fermentation is to facilitate the proliferation and predomination of desirable microorganisms in raw plant materials. The desirable microorganisms will metabolize sugars into chemicals such as lactate, ethanol, and acetate that infuse the plant

materials with various characteristics. The addition of salt and the inoculation of a defined microbial culture are the two basic methods for controlling the growth of microorganisms during fermentation. In this chapter, we will describe the predominant bacterial strains occurring in some popular fermented vegetables, illustrate their sugar metabolic reactions, and discuss how fermentation is manipulated with these organisms.

II. THE FERMENTATION OF VEGETABLES

At present only cabbage (sauerkraut and Korean kimchi), cucumbers (pickles), and olives are of real economic importance. In this chapter, the discussion is focused on these vegetable products. In addition to these vegetables, fermented carrots, the potential new products, and fermented bamboo shoots will be described.

A. Cabbage Fermentation

Sauerkraut is a fermented product made from fresh cabbage. In the cabbage fermentation process lacti acid bacteria are favored. The addition of 2.25–2.5% salt restricts the activities of undesirable gram-negative bacteria. The fermentation is started by *Leuconostoc mensenteroids*. This bacterium converts sugar to lactic acid, acetic acid, alcohol, CO_2, and other products that contribute to the flavor of sauerkraut. CO_2 helps maintain the anaerobic conditions necessary in fermenting cabbage. As the acids accumulate, *Leu. mensenteroids* is inhibited, but the fermentation continues with *Lactobacillus brevis*, *Pediococcus cerevisiae*, and finally, *Lactobacillus plantarum*. *Lb. plantarum* and *Lb. brevis* effect the final stages of sauerkraut production. *P. cerevisiae* and *Enterococcus faecalis* may also contribute to product development (1).

Kimchi is a traditional Korean fermented vegetable product. Kimchi fermentation is the Korean method for preserving a fresh and crispy vegetable texture for consumption during the winter, when fresh vegetable are not available. Although the history of kimchi fermentation in Korea can be traced to the 3rd and 4th centuries, the earliest description of the processing methods is found in 17th century works of literature (2). A fresh cabbage is cut in half or shredded, soaked in brine with an approximately 10% salt concentration overnight, and then washed and drained. The minor ingredients (garlic, red pepper, green onion, ginger) are chopped and mixed with shredded radish and stuffed between the salted cabbage leaves. The kimchi is packed in an earthen jar, buried in the ground, and pressed with a stone placed inside in order to keep the ingredients immersed in the juice. Before ripening, *Lu. mensenteroides* is the dominant microorganism, while *Lactobacillus* spp. are the major organisms in over-ripened kimchi. *Lactobacillus* species may be dominant in the later stages of kimchi fermentation depending on the temperature (2).

The difference between sauerkraut and kimchi is that of the of fermentation end-point. The best-tasting kimchi is attained before *Lb. brevis* and *Lb. plantarum* overgrowth occurs with an optimal pH of 4.5. The *Lb. brevis* and *Lb. plantarum* overgrowth diminishes the product quality, but sauerkraut production depends on these organisms.

B. Cucumber Fermentation

In the natural fermentation of pickles, selected cucumbers are placed in brine with about 5% NaCl. The brine strength is gradually increased during fermentation until it reaches around

16% NaCl. The sugars that diffuse from the cucumbers are fermented sequentially by *Leu. mensenteroides*, *P. cerevisiae*, *Lb. brevis*, and *Lb. plantarum*. Depending on the fermentation condition, about 0.6 to 1.2% lactic acid is formed in about 7 to 14 days. When the pH is lowered to 3.2, the metabolism of *Lb. plantarum* is inhibited and the fermentation is completed. In this process, the high salt level is used to protect against spoilage. The fermented cucumber must be desalted before being used in products. However, the NaCl level in the desalting solution creates a serious dumping problem. Procedures have been developed for brining cucumbers in closed anaerobic tanks at substantially lower salt concentrations (3). This approach to fermentation may allow cucumber fermentation and storage at sufficiently low salt concentration that require no desalting.

In natural fermentation, bloating in defective pickles often occurs. Bloating is due to the accumulation of CO_2 gas inside the cucumber during fermentation. The respiration of cucumber tissue and fermentation by *P. cerevisiae* and *Lb. plantarum* produces sufficient CO_2 to cause bloating (4). The degradation of malic acid to lactic acid is a major source of CO_2 when *Lb. plantarum* ferments brined cucumbers. Research has demonstrated that using a mixed culture with a malolactic-deficient mutant and normal malolactic strain of *Lb. plantarum* in brined cucumber fermentation could reduce the level of released CO_2 (5).

In cucumber fermentation, yeasts have conventionally been viewed as undesirable because it produces CO_2. However, when N_2 is used in purging cucumber fermentation tanks to prevent bloater damage, using yeast (*Saccharomyces cerevisiae* or *S. rosei*) in the mixed culture can facilitate complete sugar metabolization (6).

Softening in defective pickles is another problem. Softening is attributed to pectinolytic enzymes that degrade the cucumber tissue. The source of these enzymes may be the microorganisms growing in or on the cucumbers. To reduce fermentation defects, a controlled fermentation process is used. The controlled fermentation method employs a chlorinated brine with a 25° salinometer, acidification with acetic acid, the addition of sodium acetate, and inoculation with *P. cerevisiae* and *Lb. plantarum* (7).

C. Olive Fermentation

Olive fermentation is similar to that in sauerkraut except that the olives are soaked in a 1.6 to 2.0% lye solution before brining. The lye treatment is necessary to remove oleuropein, a bitter factor in olives. The olives are brined in containers following the complete removal of lye by rinsing the olives in fresh water. The brine concentration varies from 5 to 15%, depending on the variety and size of the olives (8). Lactic acid bacteria become prominent during the intermediate stage of fermentation. *Leu. mensenteroides* and *P. cerevisiae* are the first lactic acid bacteria to become prominent. These bacteria are followed by lactobacilli, with *Lb. plantarum* and *Lb. brevis* being the most important (9). The lye treatment may affect the microbial flora. Inoculation with *Lb. plantarum* may be required. A study has showed that using a strain of *Lb. plantarum* with the capability to produce bacteriocin as a starter controls lactic acid fermentation much better (10). The entire fermentation process may take 2 weeks to several months. The acid content of the final product varies from 0.18 to 1.27% (11).

D. Carrot Fermentation

Carrots are not a traditional vegetable for fermentation. Until 1969, carrots were fermented using a home-based process (12). Fermentation provides a simple method of preserving

raw carrots. The raw carrot slices contain a high level of reducing sugar that might cause Maillard reactions and produces dark compounds with a burnt smell during thermal processes. Using lactic acid fermentation, the reducing sugar content in the raw carrot can be decreased to a level that allows the carrot slices to be processed using high-temperature deep frying to yield chips. The deep-fried carrot chips have a light red–yellow color and pleasant taste that makes them a potential new product (13).

A mixed culture of *Lb. plantarum*, *Lb. brevis*, *P. cerevisiae*, and *Leu. mesenteriodes* is used to ferment carrots (14). Use of carrot-adapted inocula significantly reduced the lag period for early acid production despite the salt concentration. The repressive effects of increased salt concentrations on the rate of fermentation means that carrots treated with the lowest level of salt, 1.5%, require only 10 days incubation to produce a 1.0% acid level, whereas a 3.0% salt concentration requires 18 days incubation to reach a similar acid value. The acidic properties of fermented brines resemble the fermentation properties of the cabbage head brining solution (15).

A new process for carrot fermentation using an alkaline treatment with lye before inoculating a pure culture of *Lb. plantarum* was developed (16). The alkaline treatment helps inoculum establishment over the natural flora in the fermentation. However, most of the sucrose remains unmetabolized after 7 days of fermentation. Thus a long-term stability in the fermented carrots is not ensured. A high risk of secondary fermentation may present in the package product. This process was further modified using a mixed culture of *Lb. plantarum* and *S. cerevisiae* to replace the single culture of *Lb. plantarum*. The result indicated that the mixed culture was able to completely use up all of the sugars and, at same time, improve the flavor of the fermented carrots (17).

E. Bamboo Shoot Fermentation

People in the bamboo-growing regions of Asia have traditionally consumed fermented bamboo shoots. The dried Ma bamboo (*Dendrocalamus latiflorus*) shoot is a special product of Taiwan (18). Mesu is a similar product from India (19). Both are produced by using nonsalted fermentation with natural cultures.

Using mesu as a pickle and as the base for curry is a tradition in the Darjeeling hills and Sikkim area of India. A study has shown that a total of 327 strains of lactic acidbacteria, representing *Lb plantarum* and *Lb. pentosaceus* were isolated from 30 samples of mesu. These species were present in all of the raw bamboo shoot samples tested. Mesu is dominated by *Lb. plantarum* followed by *L. brevis*. *P. pentosaceus* was isolated less frequently and recovered from only 40–50% of the mesu samples. Fermentation is initiated by *P. pentosaceus*, followed by *L. brevis*, and finally succeeded by *L. plantarum* species. During the fermentation, the titratable acidity increased from 0.04 to 0.95%, resulting in a decline in pH from 6.4 to 3.8 (20). Ma bamboo shoots are fermented using a traditional natural culture. After 10 days of fermentation, the fermented bamboo shoots contain about 109 cfu/g of lactic acid bacteria, and 10^4–10^6 cells/g of yeast and mold. The final pH was 3.3 to 4.1, and the titratable acid was 1.05–1.20% (19).

III. FERMENTATION TECHNIQUES

The procedures for vegetable fermentation are varied and complicated. Basically, vegetable fermentation can be considered as a three-staged process.

A. Stage 1: The Pretreatment Steps

In this stage, the common operations include sorting and grading raw vegetables, cleaning the selected vegetables, specific pretreatment, such as peeling carrots, blanching green beans, shredding cabbage, or lye-treating olives.

B. Stage 2: The Fermentation Environment Adjustment Operation

Adding salt and inoculating the defined starter culture are two methods to set up a suitable environment around the vegetables to allow the desirable microflora to proliferate and predominate. Salt addition is necessary in most kinds of vegetable fermentation. The major contributions of salt are to inhibit the growth of pathogens and destructive spoilage microorganisms, to exert a selective effect on the microorganisms present on vegetables, to enhance the release of tissue fluids from the fermenting vegetables, and to impose a special flavor on the fermented vegetables. The amount of salt used depends on the particular vegetables. In the fermentation of cucumbers and olives, the salt concentration is 5–8% at equilibrium. For cabbages, the salt concentration is less than 2.5% at equilibrium. The difference in salt concentration between that used in sauerkraut fermentation and that used in pickle fermentation probably accounts for the difference in the types of lactic acid bacteria that grow in each fermentation environment (21).

The application of a defined starter culture is an another method of facilitating the predomination of desirable microflora in the fermenting vegetables. The lactic acid bacteria used for this purpose include *Lactobacillus* species (*Lb. plantarum* and *Lb. casei* are the most often used.), *Lactococcus lactis*, and *Leu. mensenteroides*. The defined starter cultures are capable of growing rapidly and are highly competitive under the environmental conditions used to ferment products.

C. Stage 3: The Vegetable Fermentation Process

Temperature, pH value, and anaerobiosis maintenance are major factors that influence the course of fermentation. The temperature range for vegetable fermentation is 16 to 35°C. Vegetables fermenting at 10°C lead to good quality products. Usually, the optimal temperature is between 15 and 20°C. Various microorganisms may dominate a mixed fermentation depending on the temperature. For sauerkraut fermentation, the preferred temperature is 18°C or lower. The predominant strain *Leu. mensenteroides* grows optimally at a lower temperature than the homofermentative *Lb. plantarum*, presumably resulting in a higher ratio of volatile to nonvolatile acids than at higher temperatures. For cucumber fermentation, the predominant cultures of *P. pentosaceus* and *Lb. plantarum* are capable of rapid growth at 18°C (22). The optimal temperature for vegetable fermentation depends on the predominant cultures during the fermentation.

The buffering capacity of the vegetable affects the extent of proliferation of the predominant culture used to ferment the natural sugars. Several methods have been adopted to maintain the pH during fermentation. Sodium acetate (23) and calcium acetate (24) have been used as buffering agents to assure complete sugar utilization during the primary fermentation of cucumbers. Acid neutralization during fermentation with a pH controller has also been used to assure complete sugar utilization (25). In the fermentation of carrots, sodium hydroxide treatment of peeled and trimmed carrots is a useful alternative to pasteurization to achieve controlled fermentation. Subsequent neutralization of the NaOH by adding acetic acid to the brine could lead to the formation of a buffer system in the brine.

The buffer system benefits greater utilization of the fermentable sugars by the starter culture (26). For preserving fermented vegetables for long periods of time, the pH should be controlled below 4.0 (27).

During fermentation, to maintain anaerobic conditions the plant materials must be totally covered by the brine in the vessels. Open filled vessels are normally covered with plastic sheets or wooden plates weighted down with stones or heavy matter to exclude oxygen from the air. For cucumber fermentation, anaerobic tanks provide more suitable anaerobic conditions (23). Anaerobic tanks replaced open tanks in the olive fermentation industry of the USA and Spain many years ago (28).

IV. VEGETABLE FERMENTATION MICROORGANISMS

Fresh plant material harbors numerous and varied types of microorganisms. The microflora in vegetables and fruits is largely made up of *Pseudomonas* spp., *Erwinia herbicola*, *Flaeobacterium*, *Xanthomonas*, and *Enterobacter agglomerans* as well as various molds. Lactic acid bacteria, such as *Leu. mesenteroids* and *Lactobacillus* spp. are also commonly found, as are several species of yeasts (29). Between 40 and 75% of the bacterial flora in peas, snap beans, and corn was shown to consist of leuconostocs and streptococci, whereas many of the gram-positive, catalase-positive rods resembled corynebacteria (30,31). An analysis of 30 different samples of white cabbage from four growing seasons has shown that the microflora normally is dominated by aerobic bacteria (e.g., pseudomonads, enterobacteria, and coryneforms) and yeasts, while lactic acid bacteria represent 0.15 to 1.5% of the total bacterial population (32). Vegetable fermentation involves controlling specific microorganisms or a succession of microorganisms that dominate the microflora in vegetables. Although lactic acid bacteria are present as a small population, the metabolic activities of this microorganism are indispensable in the vegetable fermentation process. Lactic acid fermentation is the most important contribution to the fermentation of vegetables.

A. The Major Lactic Acid Bacteria in Vegetable Fermentation

The major lactic acid bacteria involved in vegetable fermentation are located in three genera, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. Among the lactobacilli, several species and strains have been isolated from fresh vegetables. These include the homofermentative species *Lb. plantarum*, *Lb. casei*, *Lb. arabinosus*, and *Lb. homohiochii*, and the heterofermenters *Lb. brevis*, *Lb. fermentum*, and *Lb. buchneri*. The genus *Pediococcus* comprises two species, *P. pentosaceus* and *P. acidilactici*. Currently, *Leuconostoc* comprises a single species, *Leu. mensenteroides* (33).

The lactic acid bacteria share some common features: they are gram-positive; mesophilic, but some can grow at temperatures as low as 5°C or as high as 45°C; growing at pH 4.0–4.5 (some are active at pH 9.6 and others at pH 3.2); generally weakly proteolytic and lipolytic and require preformed amino acids, purine and pyrimidine bases, and B vitamins for growth; do not contain a citric acid cycle or a cytochrome system so no energy, is derived from oxidative phosphorylation, but energy is obtained via substrate level phosphorylation during the fermentation of sugars into lactic acid, ethanol or acetate, and CO_2.

There are four important species of lactic acid bacteria associated with vegetable fermentation: *Leu. mensenteroides*, *Lb brevis*, *P. pentosaceus*, and *Lb plantarum*. These species are successively predominant during sauerkraut fermentation in the approximate

order listed (7). *Lb brevis*, *P. pentosaceus*, and *Lb plantarum* have also been reported to ferment cucumbers (34) and olives (35). The properties of these four species are described as follows.

1. *Leu. Mensenteroides*

The colorless bacterial cell is spherical or egg-shaped and appears usually in pairs. The size of the bacterium is 0.5–0.7 μm. *Leuconostoc* is distinguished among the lactic acid bacteria in being heterofermentative and also in lacking aldolase, a key enzyme in glycolysis. Under anaerobic conditions, this bacterium metabolizes glucose via the phosphoketolase pathway and produces D-lactate. At the temperature range of 20 to 25°C, this bacterium produces dextrans from sucrose. This bacterium is capable of metabolizing citrate into CO_2 and diacetyl, which is an important flavor component in many dairy products.

2. *Lb. Plantarum*

Lb. plantarum is the final and predominant lactic acid bacterium species at the completion of fermentation in many vegetables. This is attributed to its metabolic diversity and its tolerance for low pH conditions. The optimal growth temperature for this bacterium is 30°C. The bacterial cell is a short to medium rod usually single, but sometimes in pairs or short chains. The size of the bacterium is 0.9–1.2 (width) × 3–8 (length) μm. This bacterium is classified as a facultative heterofermenter according to the metabolism of hexoses (36). It possesses both aldolase and phosphoketolase. Its homofermentative action on glucose with aldolase results in producing up to 3.5% DL-lactate. The lactate can be further metabolized to acetoin, formate, and acetate under certain conditions. In the heterofermentation of pentose via the phosphoketolase pathway, this bacterium produces lactate, acetate, and CO_2. Strains are often adopted as acid producers in starter cultures. This bacterium is the first species recognized to possess the unique ability to protect against oxygen-free radicals by a nonenzymatic superoxide reduction mediated by manganese (37).

3. *Lb. Brevis*

This bacterium has a short rod shape, occurring singly or in short chains. The size of the rod cell is 0.7–1.0 (width) × 2.0–4.0 (length) μm. The optimal temperature for growth is 30°C. This bacterium is heterofermentative and metabolizes glucose to DL-lactate, ethanol, acetate, and CO_2. This bacterium is able to reduce fructose to mannitol.

4. *P. Pentosaceus*

This is a spherical bacterium, occurring in pairs, tetrads, or clusters. The size of the coccus is 0.8–1.0 μm in diameter. This bacterium cannot grow at temperatures over 45°C. The optimum growth temperature is in the range of 28–30°C. The optimum and final pHs are 6.5–6.0 and 4.0, respectively. This bacterium is homofermentative and produces DL-lactate from glucose. Most strains ferment arabinose, ribose, maltose, fructose, galactose, and glucose to produce DL-lactate. Strains that are capable of fermenting xylose and lactose are known. Some strains produce bacteriocios during fermentation.

B. The Lactococci in Vegetable Fermentation

Although lactococci are not major lactic acid bacteria in the fermentation of vegetables, these bacteria support the fermentation with proteolytic activity (38) and the capability to

break down citrate (39). Recently, some strains of *Lactococcus lactis* subsp. *lactis* with the capability of producing bacteriocin were isolated from minimally processed fresh vegetable and fruit products. Some researchers have used these bacteriocin-producing lactococcal cultures as biopreservative in minimally processed fresh vegetables and fruits (40,41). Lactococci are spherical or ovoid cells that occur singly, in pairs, or as chains. They grow at 10°C but not at 45°C. They are homofermentative and produce L-lactate as the predominant end product of sugar fermentation.

C. The Yeasts in Vegetable Fermentation

Various groups of yeasts are present at the beginning of vegetable fermentation. Sometimes, these yeasts become predominant in the fermentation. For example, in the fermentation of olives, yeasts are the predominant microorganisms in spontaneous fermentation, because the polyphenol compounds in olives affect the microflora by inhibiting the growth of lactic acid bacteria but not yeasts (42). As a result, the final product has a shriveled form and a high salt content, and the principal bitterness does not disappear completely from the product. To limit the negative effects of yeast on the product quality, it is necessary to remove the polyphenols, acidify the cover brine, and lower the salt content to enhance lactic acid bacteria growth (43). It is well known that yeast can proliferate under low pH conditions, which usually inhibits the growth of lactic acid bacteria. In the spontaneous fermentation of tart carambola, which contains affluent organic acid with a pH is as low as 1.37–2.01, the yeasts *Candida pelliculosa*, *C. inconspicua*, *C. ciferrii*, and *S. cerevisiae* become predominant in the brine with 7–10% salt (44). In cucumber fermentation, yeasts can affect the fermentation by utilizing sugars that would otherwise be metabolized to lactic acid by the lactic acid bacteria. The yeasts can also utilize the produced lactic acid, raise the pH, and allow other microorganisms to grow. The yeasts produce large amounts of gas. This is associated with pickles that bloated or have hollow defects. Although fermentative yeasts have been viewed as undesirable in vegetable fermentation, yeasts may facilitate the removal of fermentable sugars. In cucumber fermentation, when N_2 is used to purge the cucumber fermentation tanks to prevent bloater damage, a selected yeast (*S. cerevisiae* or *S. rosei*) in a mixed culture with *Lb. plantarum* can help to exhaust the fermentable sugars rapidly and improve the quality of the products (6).

V. FERMENTATION BIOCHEMISTRY

Lactic acid bacteria are the dominant microflora in most fermented vegetables. Under normal food fermentation conditions, the main product from lactic acid bacteria metabolism is lactic acid with other products formed as by-products, such as acetic acid, acetaldehyde, ethanol, and diacetyl. All of these products contribute to control the growth of spoilage microorganisms and the specific flavor of the fermented products. Lactic acid bacteria are divided into two groups based on glucose metabolism end products. Those that produce lactic acid as the major or sole product of glucose fermentation are designated homofermentative. *Pediococcus*, *Lactococcus*, and some lactobacilli belong to this group (homolactics). Those organisms that produce equal molar amounts of lactate, carbon dioxide, and ethanol from hexoses are designated heterofermentative. *Leuconostoc* and some lactobacilli are heterofermentative (heterolactics). The major metabolic pathways of these organisms are described as follows.

A. Carbohydrate Metabolism

In general, approximately 75% of the solids in plants are carbohydrates. Total carbohydrates generally consist of simple sugars, starches, pectic substances, lignin, and cellulose. Cellulose, pectic substances, and lignin occur in all plants as the principal structural components of the cell walls. These structural polysaccharides contribute greatly to the characteristic texture of plant foods. These structural polysaccharides are usually not fermentable. The most common fermentable carbohydrates in vegetables are glucose, fructose, sucrose, and starch.

1. Glucose Metabolism

Glucose is the major fermentable sugar in vegetables. Homolactics metabolize glucose via the glycolytic pathway to yield pyruvate. Pyruvate is further reduced to lactic acid via the enzyme lactate dehydrogenase. The pathway that converts glucose to lactic acid is called lactic acid fermentation.

Heterolactics produce lactic acid via the phosphoketolase pathway. This pathway involves the initial splitting of CO_2 from the glucose molecule, followed by a further splitting of the resulting pentose (xylulose-5-phosphate) into two-carbon and three-carbon fragments in a phosphoroclastic reaction catalyzed by phosphoketolase, yielding glyceraldehyde-3-phosphate and acetylphosphate, respectively. The three-carbon fragment is eventually reduced to lactate in the same way as homolactics, and the two-carbon fragment is reduced to ethanol. Products other than lactate are generated, and the pathway is therefore called the heterofermentative pathway. Because lactic acid bacteria lack functional heme-linked electron transport chains and a functional Krebs cycle, they obtain energy via substrate level phosphorylation. In the heterofermentative pathway, 1 mole of ATP is produced per mole of glucose metabolized compared with 2 mol in the homofermentative pathway. Thus the fermentation of glucose via the heterofermentative pathway is only half as efficient as in the homofermentative pathway.

2. Fructose Metabolism

Fructose is the second major sugar substrate for lactic acid fermentation in vegetables. Lactic acid bacteria contain fructokinase and phosphoglucoisomerase to phosphorylate fructose to fructose-6-phosphate and then isomerize to glucose-6-phosphate. In the homofermentative pathway, glucose-6-phosphate is further metabolized to pyruvate via glycolysis. The pyruvate is then reduced to lactate via lactate dehydrogenase. In contrast to homolactics, heterolactics contain mannitol dehydrogenase, which catalyzes the reduction of fructose to mannitol and oxidizes NADH under anaerobic conditions (45). In this reaction a small amount of fructose is used as an electron acceptor with the remaining fructose converted to lactate, ethanol, acetate, and CO_2 (46).

3. Sucrose Metabolism

The sucrose content is less than the glucose and fructose content in most vegetables. The metabolism of sucrose in vegetable fermentation is usually incomplete in the final stage. For example, when beets containing 3.8% sucrose were fermented using *Lb. plantarum* for 12 days, the fermented beets still contained 2.8% residual sucrose. Using the same starter to ferment carrots for 35 days, 1.96% of the sucrose was reduced to 0.91%, but the glucose and fructose in the fermented carrots were exhausted at the same time (47). Sucrose

does not seem to be an optimal fermentable sugar for lactic acid bacteria. Actually, only a few lactic acid bacteria possess the ability to ferment sucrose. A screen test report showed that among 14 strains of lactic acid bacteria, *Lb. cellobiosus* appeared to be the only species that metabolizes all of the sucrose in green bean juice. Under the same conditions, *Lb. buchnerii*, *Lb. fermentum*, and *Leu mensenteroides* ferment about half of the sucrose in green bean juice (48). These lactic acid bacteria are able to hydrolyze sucrose with β-glucosidase. These products, glucose and fructose, can be metabolized via the pathways previously mentioned.

4. Starch Metabolism

The starch content in most fermented vegetables is limited, hence the amylolytic ability of lactic acid bacteria is a characteristic with little demand. Although hydrolyzing starch to simple sugars is not important in traditional fermented vegetables, a few amylolytic lactic acid bacteria have been isolated from starchy raw materials. An investigation of Mexican pozol, a fermented maize dough, indicates that lactic acid bacteria accounted for 90–97% of the total active microflora. Strains of lactic acid bacteria were isolated and identified, including *Leu mesenteroides*, *Lb. plantarum*, *Lb. confusus*, *L. lactis*, and *L. raffinolactis* (49). From sour cassava starch fermentation, *Lb. plantarum* and *Lb. manihotivorans* were isolated. *Lb. manihotivorans* grows and converts starch into lactic acid more rapidly and efficiently than *Lb. plantarum* (50,51). Durinh fermentation, these amylolytic lactic acid bacteria degrade the starch first, and then the resulting sugars allow a secondary flora to develop. An acidophilic starch hydrolyzing enzyme secreted from a strain of *L. plantarum* was isolated and partially purified. This enzyme has a molecular mass of approx. 230 kDa and is capable of hydrolyzing soluble starch, amylopectin, glycogen, and pullan. The major reaction products from soluble starch were maltotriose, maltotetraose, and maltopentaose. These reaction products suggest that this enzyme may hydrolyze both α-1,6- and α-1,4-glucosidic linkages (52).

B. Organic Acid Metabolism

Citrate and malate are the most abundant organic acids in plants. Citrate metabolism is important in fermented dairy products, while malate metabolism is important in wine.

The organisms responsible for citrate metabolism in starter cultures are leuconostoc and Cit^+ lactococci. Citrate is hydrolyzed to oxaloacetate and acetate by citrate lyase. Citrate lyase is inducible in leuconostocs and constitutive in Cit^+ lactococci (53). The oxaloacetate is decarboxylated to pyruvate, which can undergo several further transformations to diacetyl, acetoin, and 2,3-butylene glycol (54).

Malic acid is fermentable by lactic acid bacteria. Both homolactics and heterolactics are able to decarboxylate malic acid to lactic acid and CO_2. Minimal CO_2 production has been considered beneficial in maintaining anaerobiosis in sauerkraut. In cucumber fermentation, CO_2 production causes bloater damage. The decarboxylation of malic acid is undesirable in cucumber fermentation. *Lb. plantarum* produces most of the CO_2 during cucumber juice fermentation via the decarboxylation of malic acid (55). Strains of *L. plantarum* that do not decarboxylate malic acid (MDC^-) might improve cucumber fermentation. Some MDC^- mutants have been obtained through *N*-methyl- *N'*-nitro-*N*-nitrosoguanidine mutagenesis of MDC^+ parent strains. These mutants did not produce significant amounts of CO_2 when they fermented cucumber juice containing native malate (56).

C. Biogenic Amine Biosynthesis

Fermented vegetables usually contain small amounts of biogenic amines. An excessive intake of biogenic amines may cause food poisoning. Biogenic amines may also be considered carcinogens because of their ability to rect with nitrites to form potentially carcinogenic nitrosamines (57). Most biogenic amines present in fermented vegetables are formed by the action of microorganisms through the decarboxylation of amino acids during fermentation. Sauerkrauts and sauerkrautlike products are popular fermented vegetables in many countries. Thus sauerkraut could represent an important source of biogenic amines in daily diets. The bigenic amine content in commercial sauerkraut products is approximately 540 mg/kg (58). The main biogenic amines in sauerkraut are histamine, tyramine, putrescine, and cadaverine derived from histidine, tyrosine, omithine, and lysine, respectively. Biogenic amine formation in the initial stage of a spontaneous fermentation is correlated with the growth of *Leu. mensenteroides*. *Pediococcus* species are also responsible for the formation of biogenic amines. It was observed that the production of histamine was associated with the vigorous growth of the Pedicoccus species (32). *Lb. plantarum* starter cultures were able to suppress the formation of tyramine, putrescine, and cadaverine by raising the pH to impede the growth of biogenic amine producers (59). Hence it is possible to decrease the biogenic amine content in fermented vegetables by using lactic acid bacteria inoculates.

VI. STARTER CULTURE IMPROVEMENT

In vegetable fermentation starter cultures with certain beneficial bacteria are desirable. Cultures with desirable characteristics can be achieved through genetic modifications. Artificial mutagenesis and DNA recombination techniques are two available methods to generate genetically modified strains.

References on using mutagenesis to improve starter cultures for vegetable fermentation are rare. One (60) of these researches describes procedures to obtain *L. plantarum* mutants that have lost the ability to decarboxylate malic acid (MDC^-) from the parent strain (MDC^+). In this research, the parent strain was mutagenized with *N*′-methyl-*N*′-nitro-*N*′-nitrosoguanidine, and the derived mutants were then screened with designed media to select the MDC- mutants (60). The MDC- mutants do not produce CO_2 from the degradation of malic acid. When using MDC- culture in cucumber fermentation, the cucumbers were shown to be less susceptible to bloating (61). Another research involving mutation involved cultivating variant *L. delbrueckii* strains that were able to tolerate a high concentration of lactic acid (62). Treatment with ethyl methanesulfonate mutated the parent strain. The lactic acid tolerant mutants were then selected using an acclimation and selection procedure. This procedure was reported to be successful in consistently producing stable mutants with enhanced lactic acid production capacity.

Recently the DNA recombinant technique has superseded classic mutagenesis in the field of industrial strain improvement. In industrial food fermentation, new genetic techniques have already been applied to lactic acid bacteria to generate desirable starter cultures (63). No customized genetically modified strains for vegetable fermentation have yet been developed. However, some *Lb. plantarum* strains used as grass silage starters have been genetically modified by introducing heterologous genes to gain desirable attributes. Because *Lb. plantarum* is also a dominant microorganism in the fermentation of vegetables,

the genetic modification of the silage starter *Lb. plantarum* will be a useful model for cultivating desirable vegetable fermentation cultures in the future.

It has been customary to add soluble carbohydrate to silage to facilitate rapid fermentation. If a starter with the ability to hydrolyze cellulose is used in the fermentation of silage, rapid fermentation might be achieved even without adding carbohydrates. According to this thinking, a genetically modified *Lb. plantarum* with the ability to degrade cellulose was cultivated. An *Lb. plantarum* strain was transformed by inserting the ce/E gene coding endoglucanase from *Clostridium thermocellum* (64). A transformed *Lb. plantarum* strain possessing endoglucanase activity may be useful in improving the fermentation of olives and cabbage by producing acids rapidly, because this strain is able to supply mono- and disaccharides through the hydrolysis of cellulose.

A combination of polysaccharides metabolism and lactic acid fermentation trait is desirable for a starter strain to ferment plant material. For this objective, an *Lb plantarum* silage starter strain was transformed by electroporation with plasmids containing an α-amylase gene from *Bacillus stearothermophilus* and an endoglucanase gene from *Clostridium thermocellum* (65). The transformed *Lb plantarum* is a purely cellulolytic and amylolytic silage starter bacterium with the ability to produce lactic acid from the fermentation of cellulose and starch materials.

Increasing ethanol levels in lactic acid fermentation may be valuable in developing vegetable juice products. A strain of *Lb. plantarum* deficient in both D- and L-lactate dehydrogenase activity was constructed by using a two homologous-recombination processes (66). Following cloning, an alcohol dehydrogenase gene and a pyruvate decarboxylase gene originating from *Zymomonas mobilis* in this lactate dehydrogenase-negative strain resulted in an ethanol production of more than 400 mM (almost 2%).

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Chinese Pickles: Leaf Mustard and Derived Products

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I. INTRODUCTION

Various leaf mustard cultivars (*Brassica juncea* Coss) are grown in Asia, India, and Africa. In southeastern China and Taiwan, leaf mustard is commonly grown in the fall and winter seasons when temperature and humidity are lower than in the other seasons. In Taiwan, the head-type mustards (Fig. 1) are cultivated as a winter crop following the fall crop of rice, and the cultivation period is about 3 months. Most of the harvested leaf mustards are dry-salted in wells or vats for fermentation to prepare leaf mustard pickles. After fermentation, the resultant yellowish pickles, bearing a crispy texture and a sound pickled flavor, are called Hum-choy, which means sour vegetable or salty vegetable in Chinese (Fig. 2). The products are popularly accepted as pickles and ingredients for Chinese food preparation. In this chapter, preparation of leaf mustard pickles and their derived products are described.

A. An Indigenous Means of Vegetable Preservation

The use of salt to preserve foods is part of our human heritage. Production of vegetables usually depends on season with appropriate climate and geographic situation. Brine fermentation is a traditional means of preserving harvested perishable and palatable products. The brine fermentation involves complex microbial, chemical, and physical reactions and gives the final products unique flavor characteristics. Mass-produced vegetables that are fermented include cabbage, leaf mustard, turnips, hot peppers, and a variety of other green produce (1). Almost all vegetable substances, whether they are leafy, tuberous, or fruits containing seeds, provide sufficient nutrients for the growth of fermentation-related microorganisms (1,2). The growth of certain fermentative microorganisms during brine fermentation results in sensory changes that are desired by their consumers.

B. Origin of Leaf Mustard Pickles

The origin of leaf mustard fermentation is hard to trace. It is generally believed that the origin is in the Orient. When Emperor Chin Shih Huang was constructing the Great Wall of

Figure 1 Mature head-type mustards pior to harvest for leaf mustard pickle fermentation.

China in the third century B.C., a portion of the coolies' rations consisted of a fermented mixture of vegetables, probably mustards, radishes, turnips, cabbages, cucumbers, beets, and other vegetables (1). When salt is introduced and mixed with leaf mustards, it has been observed that the withdrawn brine becomes cloudy and the product acquires an acidic and pleasant flavor and aroma. The unique characteristics of the salt-preserved vegetables have been inherited for generations.

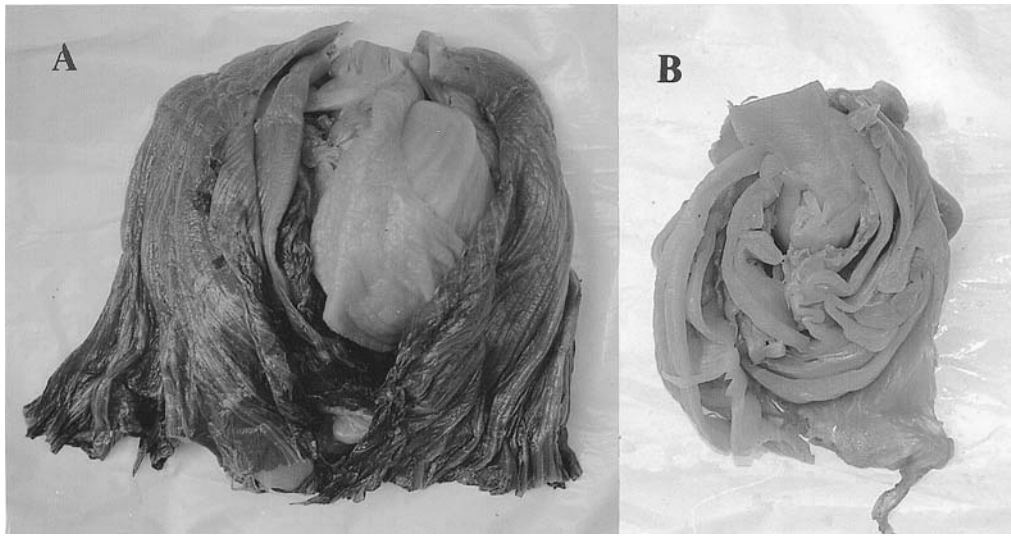

Figure 2 Leaf mustard pickles: (A) whole pickle; (B) inner head of leaf mustard pickle.

Figure 3 Fu-choy in bottles.

Figure 4 Mei-kan-choy made by dehydration of the outer leaves of leaf mustard pickles.

When the fermented leaf mustards are further sun-dried in order to reduce weight and volume, the dehydrated products are stable and convenient for storage and carrying during traveling. During storage of the dehydrated products for a prolonged period, additional unique flavor and aroma have been generated. Fu-choy (Fig. 3) and mei-kan-choy (Fig. 4) are two typical products of this type.

C. Microbiology of Leaf Mustard Fermentation

The microbiology of leaf mustard fermentation is similar to that of fermented vegetables popular in the West, such as sauerkraut and cucumber. The microorganisms responsible for the fermentation are lactic acid bacteria (LAB), *Leuconostoc mesenteroides* and *Lactobacillus* spp., and *Pediococcus* spp. (1,2). Usually, when the leafy vegetable is packed with dry salt or in brine solutions, soluble nutrients are withdrawn and support the growth of LAB. The fermentation is initiated by *Leuconostoc mesenteroides* and continued by the other LAB species. When leaf mustards were fermented with 6, 9, 12, 15, and 18% NaCl, the populations of the acid forming bacteria varied as affected by salt concentration nd time interval. Growth of LAB was inhibited when fermented with 15 and 18% NaCl (3).

II. PREPARATION AND FERMENTATION OF LEAF MUSTARD PICKLES

The use of salt stock enables processors to handle large quantities of leaf mustards for fermentation and preservation within a rather short harvest time. The term brining is often used synonymously with the term fermentation, probably due to brining being an important step in fermenting vegetables. However, brining of vegetables can be done without eventual fermentation, in particular at a fairly high salt concentration, such as salt stock. In general, fermentation is desired for most vegetables that are given a brine treatment. The fermenting of vegetables serves two main functions. First, the growth of certain fermentable microorganisms results in sensory changes bearing unique characteristics to the vegetables. These changes are desirable by those consumers accustomed to eating fermented vegetables. A second and more important function of fermentation is its preservative effect. This is accomplished when fermentative microorganisms utilize most of the fermentable carbohydrates, thereby making these carbohydrates unavailable for spoilage organisms, especially human pathogens (4).

A. General Processing Steps of Leaf Mustard Fermentation

The steps involved in fermentation of most vegetables are basically similar to one another, although specific treatments may vary depending on a particular vegetable or desired finished product. The general processing steps of leaf mustard pickles are given in Fig. 5.

The mature leaf mustard (Fig. 1) is cut and inverted in the fields for wilting for a day. The wilted leaf mustards are then trimmed and shipped to fermentation wells, usually constructed underground on the roadside, or vats (Figs. 6 and 7). Prior to deposition of the leaf mustard, the base of a well is spread with dry salt, and the first layer of leaf mustard is placed in an upright position. For the following layers, leaf mustard is deposited at an inverted position, and each layer is spread with dry salt and pressed tightly. At the top of a well, the leaf mustard is covered with a heavy-duty plastic film and weights (Fig. 7A). After about 3 days, water is drawn out of the vegetable tissues by high osmotic pressure created by the added salt. The level of leaf mustard in the wells is lowered. Further depositions of leaf

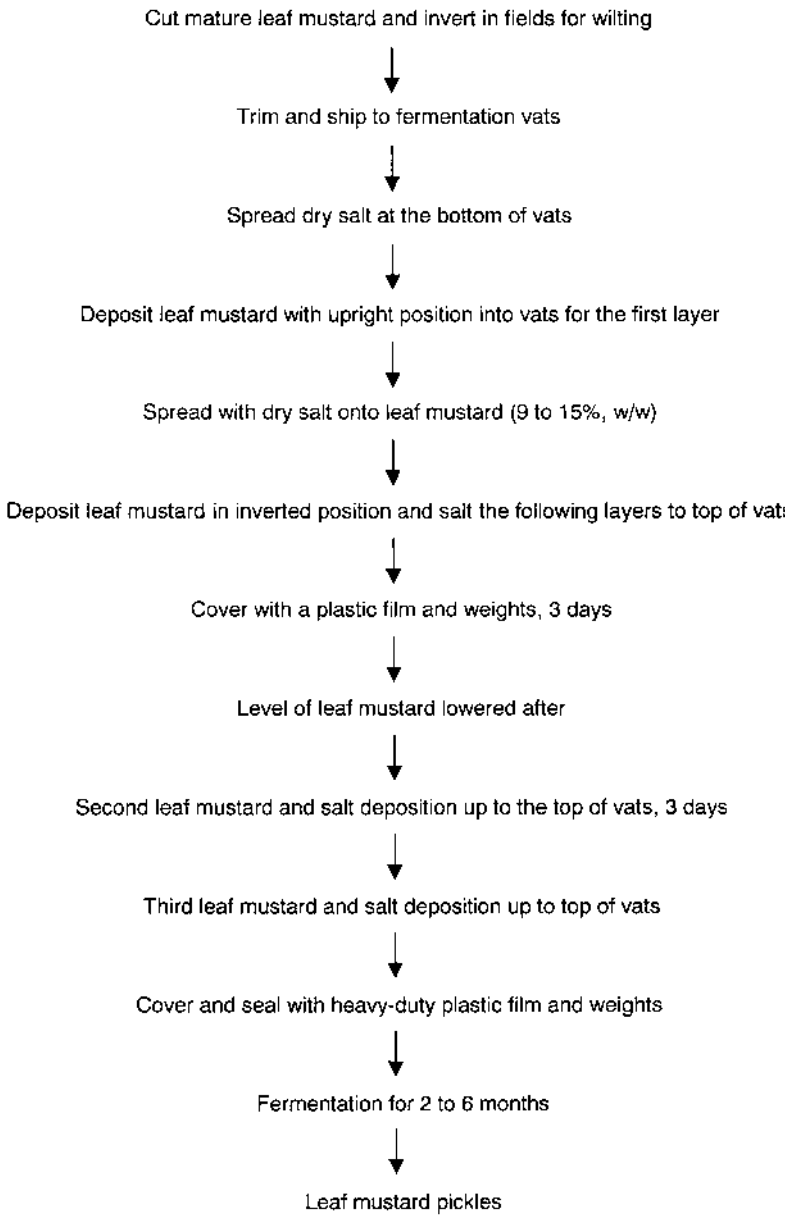

Figure 5 A general flow chart of leaf mustard pickle fermentation.

mustard and salt are repeated two or three times and finally covered and sealed with a heavy-duty plastic film and pressed with stones for long-term fermentation (Fig. 7B). The method used and the concentrations of salt needed for proper fermentation may vary mainly depending on fermentation intervals. In general, the longer the fermentation interval, the higher the salt content added. The product can be either marketed for consumer demand or hermetically sealed and sterilized in cans or pouches for local and overseas marketing.

Figure 6 Vats for processing leaf mustard fermentation.

B. Changes During Fermentation

During leaf mustard fermentation, a variety of microorganisms may develop. In the initiation stage, LAB species involve competition with other microorganisms for fermentable carbohydrates and eventually predominate, with a resultant drop in pH value and the exclusion of undesirable bacteria. When the leaf mustard was fermented with 6, 9, 12, 15, and 18% NaCl for 30 days, pH values changed from the initial value of 5.3 to 3.5, 3.5, 4.8, 5.0, and 5.2, respectively (3). In addition to fermentable carbohydrates, the contents of crude fiber, crude protein, free amino acids, and water-soluble vitamins including thiamine, riboflavin, and niacin were higher in leaf mustard pickles fermented with 15 and 18% NaCl than in pickles fermented with 9 and 12% NaCl (5). When leaf mustard was fermented with 6 and 9% NaCl for 20 days to reach the highest level of microbial population, the total populations, mainly LAB, were 3.2×10^7 and 1.2×10^7 CFU/mL, respectively. However, when leaf mustard was fermented with 12, 15, and 18% NaCl for 30 days, the microbial populations were 5.0×10^7, 4.6×10^7, and 4.6×10^7 CFU/mL, respectively (3). In addition to LAB, the growth of halophilic yeasts can also prevent further spoilage by helping to exhaust the fermentable carbohydrates in the production of ethanol. The formation of ethanol may be responsible for improving the flavor of fermented vegetables.

III. PRODUCTS: FU-CHOY AND MEI-KAN-CHOY

Various derived products from leaf mustard pickles are common in the Orient. Fu-choy (Fig. 3) and mei-kan-choy (Fig. 4) are two of the most popular products. Fu-choy is a

Figure 7 Mustards are deposited and spread with dry salt into wells for fermentation. (A) Mustards are deposited into wells and pressed by weights; (B) mustards are sealed and pressed by stones for fermentation.

product that went through an in-container secondary fermentation, whereas mei-kan-choy is an intermediate moisture fermented product. The schematic flow chart of processing is shown in Fig. 8.

The initial procedure is the same as that of leaf mustard pickle preparation. After fermentation, the leaf mustard pickle is divided into inner heads (Fig. 2B) and outer leaves and subjected to solar drying. For large-scale production, the whole pickles are cut vertically through the stems and hung on bamboo sticks for sun drying (Fig. 9).

A. Fu-choy

For fu-choy production, the fermented mustard leaf is used as the raw material. After partial dehydration, the stems and inner leaves are cut into thin stripes (ca. 1 to 2 cm in thickness

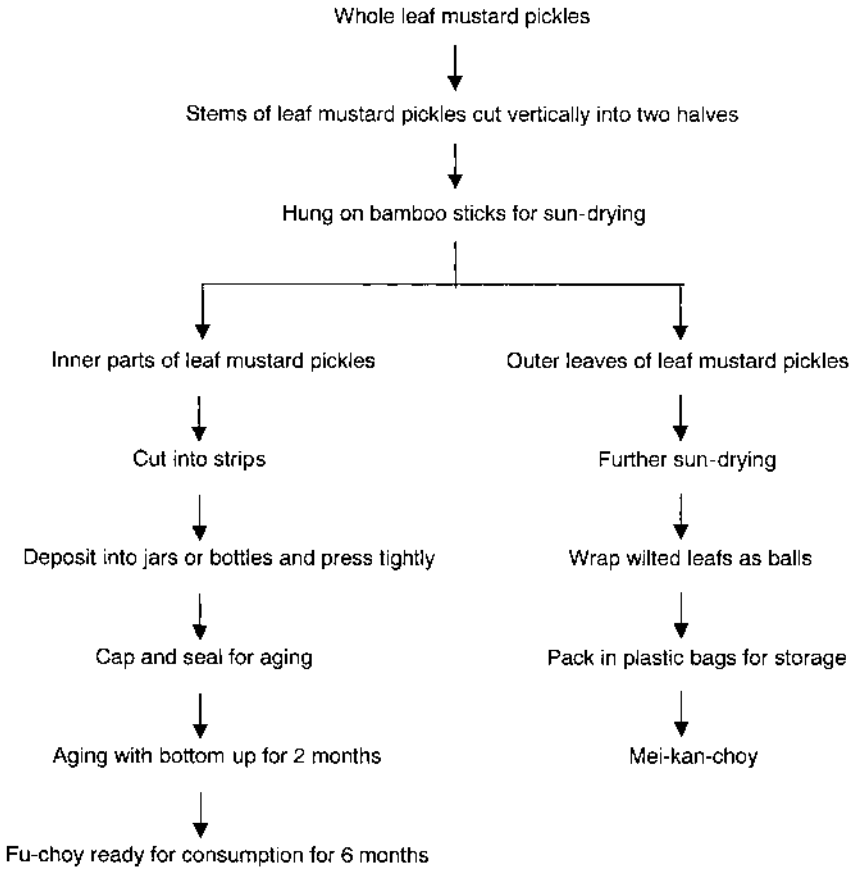

Figure 8 A general flow chart of fu-choy and mei-kan-choy preparation.

and 5 to 9 cm in length). The stripes are packed tightly in glass bottles (homemade styles) or various types of jars (Fig. 3). The neck of the container is filled with the partially dried leaves originating from the outer leaves and followed by sealing with caps or plastic films tied with ropes. The bottles or jars are aged in a bottom-up position for 2 to 3 months. During this period, unique flavor and aromas are developed. The products are stable at ambient temperature and can be stored and consumed for several months to a year.

B. Mei-kan-choy

Mei-kan-choy (Fig. 4) is usually made from the sun-dried outer leaves of leaf mustard pickles. After fermentation, the outer leaves are removed for sun drying or removed after sun-drying of the whole leaf mustards. The dried outer leaves are wrapped into bunches, packed, and stored at ambient temperature. After rehydration and cleaning, the leaves are commonly chopped into small pieces to cook with meats. In particular, it is an important ingredient to cook with pork slices or ground pork, such as mei-kan-ko-lo, a famous Chinese dish.

Figure 9 A large-scale sun-drying of leaf mustard pickles in preparation of fu-choy and mei-kan-choy.

IV. PROCESSING INNOVATIONS OF LEAF MUSTARD PICKLES

Homemade leaf mustard fermentation has continued for centuries with little standardization, and the household methods are generally passed on from one generation to the next. There was little standardization of practice until the early period of the 20th century. The application of science to pickling and identification of the bacteria and yeasts present in fermenting vegetable substances in the early 1900s was a milestone of vegetable fermentation (1). Advancements in production methods and the development of superior and disease-resistant strains of vegetables have been largely responsible for providing adequate vegetables for preservation. The major improvements in vegetable fermentation during the past 50 to 60 years began with the development in microbiological science initiate about 100 years ago. These culminate with the conclusion that the addition of salt creates a specific environment and allows more than one species of LAB to contribute to leaf mustard fermentation.

A. Modified Dry-Salt and Brine Fermentation

A modified procedure for quality improvement of leaf mustard pickling was reported by Chen and Lee (6). Prior to depositing into vats, the leaf mustard was cleaned with water to remove dust and foreign materials. Based on the weight of fresh leaf mustard, 9 to 12% NaCl (w/w) were mixed and deposited into vats for 3 days. Then the partially dehydrated leaf mustard was replenished with 20% (v/w, based on the original leaf mustard weight) of the same salt solution (9 to 12% NaCl) to cover the leaf mustard. After tight sealing of the container and subject to fermentation at ambient temperature for 2 to 5 months, sound pickles are produced (5,6).

B. Low-Salt Fermentation of Leaf Mustard Pickles

In the conventional fermentation of leaf mustard pickles, waste disposal of the brine solution left in the fermentation wells or vats has been an environmental concern. Low-salt fermentation, the recovery of salt, and reusing the brine solution are possible options. A technique for the reduction of salt used in leaf mustard fermentation has been developed (7). Leaf mustard was dry-salted and fermented with 9% salt for 1 week. The uncured leaf mustard was hydraulically pressed to remove 60% of weight. Then the partially dehydrated leaf mustard was shredded and supplemented with 3% of dry salt and canned for further fermentation at ambient temperature for an additional 6 months. After fermentation in cans, the flavor and texture of the leaf mustard pickles were fairly acceptable.

V. OUTLOOK FOR LEAF MUSTARD PICKLES

Leaf mustard pickles are indigenous fermented foods. Since ancient times, most are prepared in homemade styles in order to obtain fresh products. Quality of the products varies widely owing to inconsistency in the harvested leaf mustard and in fermentation techniques (in particular, fermentation scale, salt concentration, and fermentation interval). In recent years, small- and medium-size industries of leaf mustard fermentation and marketing channels for the products have commenced. Fermentation techniques and standardization of the products have also been improved. Products such as freshly fermented or sterilized pickles (including ball-heads and shredded slides), fu-choy, and mei-kan-choy are popular items consume in Chinese communities all over the world.

Currently and in the future, the continuous involvement of science and technology in leaf mustard production, optimization of fermentation, salt reduction, quality improvement, and enhancement of packaging and marketing is still needed. In addition to the unique pleasant pickle flavor, leaf mustard pickles containing rich edible fiber but low calorie contents renders the products favorable to the public. Based on the inevitable emergence of the global village, knowledge and technology of leaf mustard cultivation, pickling, and product features will be highlighted and of benefit to people worldwide.

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Kimchi

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I. INTRODUCTION

The word *kimchi* is the generic term for Korean fermented vegetables, which is derived from the Chinese characters pronounced *chimchae,* meaning brined vegetables. Traditionally in Korea, large quantities of *kimchi* are prepared as an annual event, *kimjang*, for eating during the winter when the fresh vegetable supply is limited. Most of the vegetables cultivated in Korea are used as sources for making kimchi. Although 161 or 187 kinds of kimchi are currently reported, depending on the varieties and preparation methods of those vegetables (1,2), the most popular kimchi is made with Korean *baechu* cabbage (known to Westerners as Chinese cabbage). The various types of kimchi are prepared through a series of processes including the pretreatment of the main vegetables, spices, and other subingredients. The ingredients used for kimchi preparation, as well as the fermentation conditions such as temperature, air, salt content, and packaging materials, are important factors to increase the preservation period, taste, and functionality of kimchi (3). Fermented kimchi contains high levels of lactic acid bacteria (LAB, $10^{7\sim9}$ CFU/mL), organic acids, and various nutrients and functional components, that result from the ingredients and the fermentation process.

Properly fermented kimchi is flavorful, having the distinct savor of a combination of sour, spicy, hot, sweet, and carbonated-fresh tastes. For the Korean people, kimchi is consumed as the most favorable and frequent side dish accompanying cooked rice. Actually, Koreans have kimchi at the everyday diet table, every meal. During difficult times when no other side dishes were available, kimchi may have been the only side dish they had. During the 1950s (the post–Korean War period), the consumption of kimchi per person, per day was 200 to 300 g, decreasing to 124.4 g in 1998. This is 44 percent of the whole consumption of vegetables (248 g) for each Korean per day (4), which is still high compared to 50 years ago when Koreans were so economically depressed that they could not afford various side dishes.

Nutritionally, kimchi is an important source of vitamins, minerals, dietary fiber, and other functional nutrients and phytochemicals. Kimchi might help to increase appetite, reduce constipation, control intestinal flora, and have anticarcinogenic and antiaging effects and other health benefits (5). In the past, kimchi was prepared as a homemade product, but large quantities of kimchi are now produced commercially. A variety of kimchis are

packaged in aluminum film bags, and plastic or glass jars for domestic consumption and for export.

II. THE HISTORY OF KIMCHI

Records indicate that salted vegetables as a type of macerated vegetable were consumed in Korea as early as the 3rd or 4th century A.D. (6). The first record of kimchi appears in the *Koguryojon* of China's *Weizdongyizhuan* region, *Samguozhi* (A.D. 289). The book states, "The Koguryo people [referring to the Korean people] are skilled in making fermented foods such as wine, soybean paste, and salted and fermented fish." This passage indicates that fermented foods were widely enjoyed at that time. According to a Korean record, the *Samkuksaki*, published in A.D. 1145 during the Silla dynasty (about A.D. 720), fermented vegetables were prepared using a stone pickle jar, indicating that these foods were commonly available at that time. During the early Koryo dynasty (A.D. 918–1392), Buddhism accepted vegetarian diets while rejecting meat consumption. The preparation and the use of various added ingredients became more diverse with time. Records show that kimchi was garnished with garlic and with spices such as Chinese pepper, ginger, and tangerine peels.

For instance, the *Kapoyukyong,* from the *Dongkukisangkukjip* (A.D. 1241), states that white radish leaves in soy paste were used to prepare summer vegetables and salt for winter vegetables, making these kimchi preparations differently from the *jangajji* (vegetables pickled in soy paste or soy sauce). Also, the text states, "prepare kimchi for winter," suggesting a traditional custom of kimjang, the fall kimchi preparation. The kimchi cited here is similar to today's radish (i.e., a large broth-containing) kimchi and other similar kimchis like nabakji and dongchimi.

The word *chimchae*, the Chinese term for kimchi, appears for the first time in Yi Saek' s *Mogunjip* (A.D. 1626). The *Dongkukisankukjip*, published in the 12th century, mentions fermented vegetables being consumed in winter. Until the Koryo dynasty, the main vegetable used to make kimchi was radishes rather than Korean *baechu* cabbages. Records also show that cucumber, eggplant, and green onions were used to make macerated vegetables at that time in addition to radishes.

During the early Choson dynasty (A.D. 1392–1600), many foreign vegetable species were introduced into Korean foods, and as a consequence, kimchi ingredients became more varied and the preparation methods more elaborate. In the Choson *Wangjoshillok* (A.D. 1409) there is mention of *chimjanggo* for the storage of kimchi. From this reference, a designated place was reserved for the kimchi for kimjang.The word kimjang originates from *chimjang*, indicating the storage of kimchi for winter.

During the mid-Choson dynasty (after A.D. 1600) many different vegetables were imported from foreign countries. Records of red peppers are first found in the *Jibongyusol* (A.D. 1613), and their use in kimchi was first recorded in the *Sallimkyongje* (A.D. 1715). Kimchi developed more complex yet harmonious tastes, with red pepper as one of the main subingredients. The number of vegetables used as main and subingredients of kimchi grew as well. Many kinds of salted and fermented fish came into use. The combined use of animal meats and vegetables in kimchi resulted in a great combination of tastes and nutrition. As the savory taste of kimchi improved, baechu cabbage and white radishes (similar to Japanese Daikon) became the main ingredients of kimchi. The most popular tongbaechu kimchi (meaning whole cabbage kimchi), evolved as a product of the 19th century. The variety of kimchi became diverse with offerings such as watery-mulkimchi, stuffed sobaegi, and mixed sokbakji kimchi.

The *Umshikdimibang* (A.D. 1670) describes the processing methods of seven types of vegetable pickles. In the *Jungbosallimkyongje* (A.D. 1776), 41 different kinds of kimchi are described, thereby making an invaluable documented record on the history of kimchi. In this book, tongbaechu kimchi was introduced, a kimchi containing meat and fish ingredients. During this time, many types of kimchis were introduced in written form for the first time in history. One type is chonggak kimchi (pony-tail radish kimchi) that was made with a small radish, using all its leaves. Precursors of today's oisobaegi (stuffed cucumber kimchi), sokbakji (radish and cabbage in mixed form), and dongchimi (broth-based radish kimchi) also made their first appearance.

In the *Kyuhapchongso* (A.D. 1815), which is considered the first encyclopedia of the Choson dynasty, sokbakji-type kimchi is described. This variety is characterisized by the use of more ingredients and a clear distinction among main components; it has secondary additives of salted and fermented fish. In the *Imwonshibyukji* (A.D. 1827) a wide variety of kimchi (97 varieties) is mentioned, emphasizing the use of red peppers for kimchi.

III. THE CLASSIFICATION OF KIMCHI AND RAW INGREDIENTS

Many types of kimchi are available depending on the raw ingredients used, processing methods, harvest seasons, and geographical regions. Although baechu (Korean cabbage) and radish are the most widely used in the making of kimchi, other vegetables are used depending on their seasonal availability. Because of the cold northern, and the mild southern Korean winters, the kimchi prepared in the north contains less salt, whereas the kimchi in the south requires more salt for long-term preservation. Also, people living near the sea naturally use more fish products in their kimchi.

Cho and Nam (7) reviewed published data on kimchi in Korea from 1959 to 1976, reporting 55 varieties of kimchi: 31 of kimchi, 8 of jangajji (sliced vegetables pickled in soy sauce), 4 of kaktugi (diced-radish kimchi), 2 of dongchimi (whole radish kimchi without red pepper), and 6 other types. In 1994, Park et al. (2) reported a total of 187 types of kimchi existing today in Korea.

Son (1) classified 161 varieties of kimchi into eight different groups based on the main raw ingredients (Table 1). Briefly, the first group comprises baechu kimchi (12 types), of which tongbaechu kimchi is the most popular, followed by baek kimchi and bossam kimchi. In the second group are radish kimchis (17 types), with dongchimi and chonggak kimchi being the favorites. In the kaktugi group (25 types), radish kaktugi is the most popular. In the fourth group composed of sokbakji and nabak kimchi, there are 20 varieties including sokbakji and nabak kimchi. Green vegetables and stem vegetables comprise the fifth classification group of kimchis (27 types), which includes got (mustard leaf) kimchi and kodulbaegi kimchi. The sixth group includes fruit- and root-vegetable kimchi (27 varieties), among them cucumber kimchi and burdock kimchi. The green onion, garlic, and leek group contains 14 varieties. Leek kimchi and green onion kimchi are favorites that are usually prepared in Kyoungnam province, in the southern part of Korea. The eighth group has 19 varieties of kimchi composed of meat, fish, shellfish, and seaweed.

A survey on the preference of kimchi prepared in Korean households shows that baechu cabbage kimchi is the most frequently prepared, followed by radish kaktugi, broth-containing dongchimi, and then miniature radish and stem chonggak kimchi (3). Thus, although both baechu kimchi and kaktugi are important kimchis, baechu cabbage kimchi is the far more popular and represents the common name of kimchi. Also, marketed kimchis are mainly baechu kimchis ($>$ 70 percent) and radish kimchis (around 20 percent).

Table 1 Classification of Kimchi by Raw Ingredients

Group	Varieties
1. Baechu kimchi (Korean baechu cabbage kimchi) (12 varieties)	Tongbaechu kimchi (whole cabbage kimchi), baek kimchi, bossam kimchi (wrapped-up kimchi), vegetables pickled right before eating, etc.
2. Radish kimchi (17 varieties)	Dongchimi, radish zangi, chonggak dongchimi, mupinul kimchi (slit-cut radish kimchi), chonggak kimchi, sunmu kimchi (turnip kimchi), chae kimchi (julienne radish kimchi), muchung dongchimi (radish leaf kimchi), musobagi (stuffed radish), pickled radish kimchi, musun kimchi (radish shoot kimchi), etc.
3. Kaktugi (25 varieties)	Radish kaktugi, radish and oyster kaktugi, radish and wild rocambole kaktugi, radish and leek kaktugi, radish and radish leaf kaktugi, cucumber kaktugi, chonggak kaktugi, radish and salted pollack guts kaktugi, parboiled radish kaktugi, yolmu kaktugi, radish and cod kaktugi, etc.
4. Sokbakji and nabak kimchi (20 varieties)	Sokbakji, wax gourd sokbakji, baby ginseng nabak kimchi, nabak kimchi (sliced cabbage and radish kimchi), chang kimchi, changzanji, etc.
5. Green vegetables and stem vegetables (27 varieties)	Shigumchi kimchi (Spinach kimchi), gat kimchi (mustard leaf kimchi), kodulbaegi kimchi (wild lettuce kimchi), kongnamul kimchi (soybean sprout kimchi), minari kimchi (dropwort kimchi), doraji kimchi (broad bellflower kimchi), dolnamul kimchi (sedum kimchi), young mustard leaf kimchi, etc.
6. Fruit and root vegetable kimchi (27 varieties)	Oi kimchi (cucumber kimchi), oi sobagi (stuffed cucumber kimchi), pickled cucumber, hobak kimchi (pumpkin kimchi), gaji kimchi (eggplant kimchi), koguma kimchi (sweet potato kimchi), putgochu kimchi (green pepper kimchi), uong kimchi (burdock kimchi), cucumber kimchi, kam kimchi (persimmon kimchi), etc.
7. Green onion, garlic, and leek kimchi (14 varieties)	Leek kimchi, green onion kimchi, green onion zanji, dalrae kimchi (wild rocambole kimchi), etc.
8. Meat, fish, shellfish, and seaweeds kimchi (19 varieties)	Meat kimchi, chicken kimchi, pheasant kimchi, earshell kimchi, green laver kimchi, oyster kimchi, codfish kimchi, dried pollack kimchi, squid kimchi, Alaska pollack kimchi, marine products kimchi, miyok kimchi (brown seaweed kimchi), etc.
Total 161 varieties	

Source: Ref. 1.

The raw ingredients used to make these kimchis are listed in Table 2. The vegetables most frequently used to make kimchi are baechu cabbage, radish, miniature radish, cucumber, etc. The spices used to prepare kimchi are green onion, garlic, red pepper powder, ginger, leek, mustard, black pepper, onion, and cinnamon. Commonly used seasoning for kimchi are salt, salted and fermented shrimp, anchovies, soy sauce, vinegar, chemical seasoning agents, sweetening agents, sesame seed or its oil, and oyster; these are optionally

Table 2 Raw Ingredients Used for the Preparation of Kimchi

Groups	Raw ingredients
Main raw vegetables	Baechu (Korean baechu cabbage), radish, pony-tail (chonggak) radish, young oriental radish, cucumber, green onion, lettuce, Western cabbage, leek, green pepper, etc.
Subingredients	
Spices	Red pepper, green onion, garlic, ginger, mustard, black pepper, onion, cinnamon, etc.
Seasoning	
Salt	Dry salt or brine solution
Salt-pickled seafood	Anchovy, small shrimp, clam, hairtail, yellow corbina, etc.
Other seasoning	Sesame seed, soybean sauce, monosodium glutamate, corn syrup, etc.
Other materials	
Vegetables	Watercress, carrot, crown daisy, parsley, mustard leaves, etc.
Fruits and nuts	Pear, apple, jujube, melon, ginkgo nut, pine nut, etc.
Cereals	Rice, barley, wheat flour, starch, etc.
Seafoods and meats	Shrimp, Alaska pollack, squid, yellow corbina, hairtail, oyster, beef, pork, etc.
Miscellaneous	Mushrooms, etc.

added to kimchi to improve and vary flavor and taste depending on the type of kimchi. Additional kimchi ingredients are fruits (jujube, ginkgo nut, pine nut, chestnut, apple, orange, etc.), cereals (polished barley, glutinous rice, wheat flour, and malt), seafoods (oyster, squid, shrimp, and Alaskan pollack), and meats (beef and pork). Fish and meats are added to improve the flavor of kimchi, and cereals are added to enhance lactic fermentation.

IV. PROCESSING OF KIMCHI

Kimchi being fermented food, preparation methods differ depending on the ingredients used, family preference, regional customs, etc. The essential process consists of maceration of raw ingredients, mixing the ingredients, packaging, and fermentation. Pretreatment of raw ingredients includes grading, washing, and cutting. Other ingredients are also graded, washed, and cut, sliced, or chopped for the proper mixing.

Pretreated raw baechu cabbage or radish is brined at proper concentrations of dry salt (natural salt prepared from seawater with removal of Mg^{2+}), brine solution, or dry salt plus brine solution. Brine-treated (i.e., macerated), rinsed baechu cabbage is mixed together with a mixture of chopped or sliced subingredients (spices, seasonings, salt-pickled fishes, and other vegetables) and dry salt to make a final salt concentration of 2.5 percent. The blended preparation is then fermented under the appropriate conditions.

The major treatments used in the making of kimchi and the important steps that can affect its quality, taste, and functionality are as follows:

A. Selection and formulation of the raw ingredients
B. Salting, rinsing, and draining
C. Pretreatment of subingredients and mixing process
D. Placement in crocks (fermentation vessels)
E. Fermentation

A. Selection and Formulation of the Raw Ingredients

The quality and species of the major ingredients (Korean baechu cabbage, *Brassica campestris* sp. Pekinensis or oriental radish, *Raphabus satirus* L.) may significantly affect the product characteristics of kimchi. For example, only carefully selected baechu are used for baechu kimchi. The best baechu fit for a high quality kimchi has a compact structure, an oval-shaped head, white tissue, and green leaves. Essential to this selection process are the evaluations among the cabbage varieties for their physicochemical and organoleptic properties (8). In addition to the quality of the raw ingredients, the cultivation method, the kind and type of the vegetables or salt-pickled seafood can also affect the fermentation behavior and quality characteristics of the kimchi.

There are two major ways to prepare baechu cabbages for kimchi. One is used for tongbaechu kimchi, a more elaborate baechu kimchi preparation using whole cabbages, preserved for the long-term winter season. The other method is used for chopped or cut matbaechu kimchi, a common preparation style for commercial products, export, and daily, informal family eating. The preparation of these two baechu kimchis is very similar, including ingredients and recipe, except for the cabbage preparation process. For the preparation of tongbaechu kimchi, graded and washed baechu cabbage is slit lengthwise into two or four parts with a knife inserted through the bottom of the cabbage head. The cabbage sections are treated with dry salt for several hours or with 10 percent brine for about 10 hours. The macerated cabbage is rinsed to remove excess salt and then drained. A premixture of spices and other ingredients, according to a given recipe, are packed between the layers of the cabbage leaves. The stuffed cabbages are then placed in a jar that allows for a facultative anaerobic condition for the fermentation. A flow chart for processing the baechu kimchi is shown in Fig. 1.

For chopped matbaechu kimchi preparation, cabbage is cut 3 to 5 cm in length, macerated in a salt solution (8 to 15 percent concentration) for 2 to 7 hours, rinsed with fresh water, and then drained. As an alternative salting method, a whole cabbage that is cut into two or four pieces is first macerated like tongbaechu kimchi before being cut into smaller sizes. Separately premixed sliced radish, cut green onion, chopped garlic, chopped ginger,

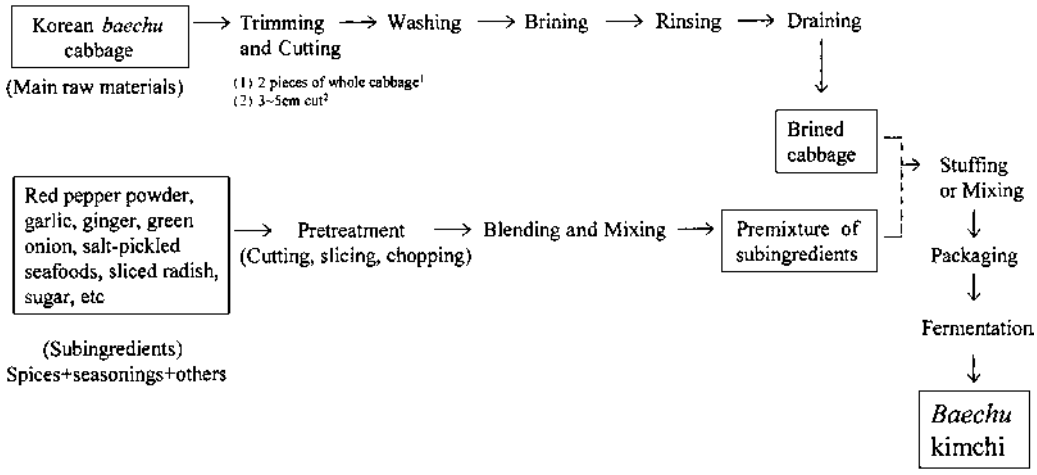

Figure 1 Flow chart for processing of baechu kimchi. (1) Tongbaechu kimchi, (2) Matbaechu kimchi.

red pepper powder, salt-pickled seafood, dry salt, etc. are combined to make a premixture of spices with other minor ingredients as directed by the recipe.

B. Salting, Rinsing, and Draining

The macerating step may be accomplished by using either a brine solution or dry salt. This treatment is the most important step for taste, texture, fermentation, and preservation. In general for baechu kimchi, brining is carried out over a wide time range (1 to 15 hours), depending on the salt concentration (5 to 18 percent) and salting temperature (8 to 25°C). Brining at a low temperature (5°C) provides better flavor than at a higher temperature (20–25°C) owing to the support of *Leuconostoc* growth during the brining. For radish kaktugi, dry salt is added to cubed radish for a given time without rinsing. However, salt-macerated cabbages for baechu kimchi are rinsed and drained before blending with the premixture of other ingredients. For baechu kimchi, the salt concentration is adjusted to 2.2 to 3.0 percent of the final product, which assures a less salty, better tasting, and crispy quality kimchi. If the concentration is less than 2.2 percent, fermentation would be too fast, which frequently causes quick acidification and/or softening of the cabbage tissue. On the other hand, if the salt concentration is higher than 6 percent, the kimchi would be too salty, have less flavor, and have a poor appearance.

Generally, the brining process sets the moisture concentration of macerated cabbage, the relative volume and weight, and the internal void space of cabbages. These changes are extremely important because the physical properties of cabbages can be markedly affected, especially for crunchiness of the tissue, which gives a typical textural freshness to the final product. Brining also reduces the content of water- or salt-soluble compounds such as Ca and Mg in the cabbage and increases the salt content in the tissue (9). Furthermore, as a result of brining, the total aerobic bacteria, yeast, and mold counts in salted cabbage are reduced; however, LAB may be increased because of the action of salt (10). It has also been suggested that salting with various commercially available products may affect growth of the microorganisms differently during kimchi fermentation. Heat-treated salt, especially bamboo salt (salt baked in bamboo), is known to reduce growth of *Lac. plantarum, Pichia membranaefaciens,* and *E. coli*, but not *Leu. mesenteriodes* (11).

C. Pretreatment of Subingredients and Mixing Process

As shown in Fig. 1, the brined baechu cabbage is spiked with premixed subingredients of spices, seasonings, and other things. Red pepper powder, garlic, ginger, and green onions are the main spices added to the kimchi. These subingredients should be selected for their good quality, as they can substantially impact the final quality of the kimchi. For instance, appropriate amounts of capsaicin, the sugar content, and the color intensity of the red pepper are important factors in selecting a powdered red pepper. The skinned garlic and ginger are crushed and finely minced. Medium-size green onions are cut into 3 to 4 cm pieces. Radish pieces for a filler for the spice mixture should be less than 1 cm in size. The following ingredients can be added to the premixture as seasoning: salt-pickled seafood, juice of fermented anchovy or small shrimp, and salt. These can added to enhance or vary the flavor and to adjust the salt level. Rice paste as a carbohydrate source can be added to boost the microorganisms for fermentation. Sesame seed or MSG can also be included as fine adjustments to improve taste.

Various other taste-enhancing materials can also be added to the subingredient premixture, based on family tradition, economic situation, and seasonal and regional avail-

ability of the ingredients. For example, watercress, mustard leaves, pear, apple, pine nut, chestnut, ginkgo nut, cereals, and various fish and meats (Table 2) can be incorporated into kimchi. For elaborate tongbaechu kimchi, premixtures of subingredients are blended and then stuffed into macerated cabbages. For the simpler matbaechu kimchi, the premixtures are simply blended with brined, cut cabbages.

As an alternative method to the conventional kimchi manufacturing process, the brined baechu cabbage and kimchi seasoning mixture (premixtures of subingredients) are prepared separately and then stored (12). The brined cabbage and kimchi seasoning mixture can be mixed as needed. The kimchi seasoning mixture can safely be stored without microbial growth. Because sensory evaluation studies proved satisfactory, this new processing method of kimchi making will be useful for households and kimchi factories, since the sensory evaluation studies were satisfactory. However, further studies are needed for this process.

D. Placement in Crocks

The premixtures of subingredients for stuffed tongbaechu kimchi are either stored for ripening or packaged for sale. Traditionally, a large quantity of cabbage heads were used for making kimchi, which were stored in underground potteries for the winter. This practice is called kimjang, and it is a major annual event for the Korean family. Kimjang kimchi is the most traditional way to make baechu kimchi, and it is made between mid-November and early December, depending on the climate of the particular year and region. This kimchi is then consumed the following spring. The freshly prepared, unripened kimchi is tightly stacked into the large earthenware crocks (Fig. 2) and covered with the remaining leaves of the brined cabbage. To maintain the cabbages in a facultative anaerobic condition, large stones are used to weigh down the pottery. The tiny pores naturally found in crocks trap air and provide the facultative anaerobic condition for endogenous microorganisms needed

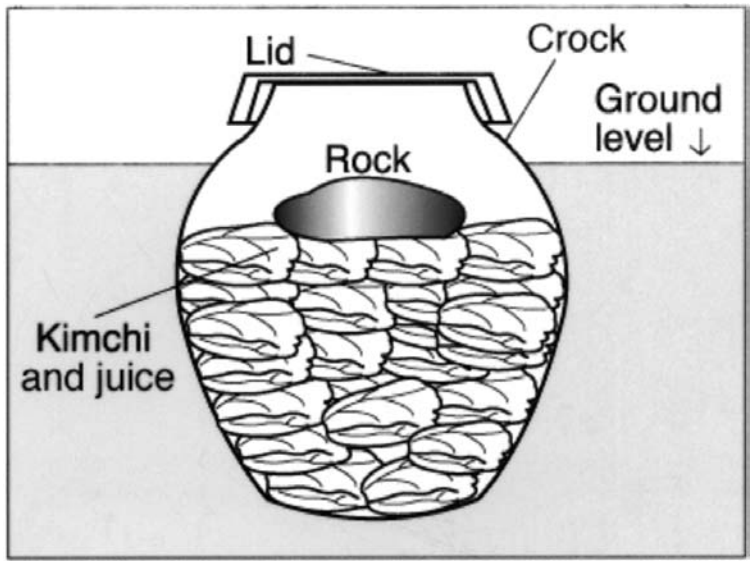

Figure 2 Traditional crock for kimchi storage that is buried in the ground.

during the fermentation. To maintain a constant temperature, crocks are buried underground (80 to 90 percent of the container's depth) and are covered with thick rice straw mats for protection from direct sunlight and cold air.

Recently, lesser amounts of winter kimchi are prepared owing to the more ready availability of ingredient items, including meats, fish, and fresh vegetables as well as commercial kimchi products. Koreans now enjoy freshly fermented kimchi any time of the year owing to the year-round availability of fresh vegetables and of common household refrigerators and commercial kimchi products. It is common for Korean families to make small batches of kimchi as needed and to store it under refrigeration for short periods to extend its freshness.

Recently, home appliance companies have developed a kimchi refrigerator. This invention provides Korean households with better fermentation and storage by permitting a programmable low temperature fermentation as well as giving a convenient storage place in which to make a better quality of kimchi.

E. Fermentation

The fermentation of kimchi occurs mainly because of the raw materials' endogenous microorganisms, especially in the brined cabbages. Although fermentation may be initiated by various microorganisms, LAB ferments sugars in the cabbage and other subingredients that gradually dominate other anaerobic microorganisms by organic acid formation. Various chemical, physical, and biological factors also may contribute directly to the growth of microorganisms and to the extent of fermentation. Several factors influence kimchi fermentation: kinds of microorganisms, salt concentration, fermentable carbohydrates, other available nutrients, the presence of inhibitory compounds, the absence of oxygen, pH, and fermenting temperature. The salt concentration, temperature, and pH have a great effect on the rate and extent of the fermentation by LAB. It takes a shorter time to make optimally ripened kimchi when the temperature is increased and the salt concentration is decreased. Detailed microbiological characteristics and fermentation and preservation methods for kimchi are discussed in Sec. V.

F. Processing Methods for Major Kimchi

As mentioned, there are 161 to 187 varieties of kimchi; baechu kimchi is the most popular, and radish kaktugi and dongchimi are also frequently prepared. The processing methods for these three representative kimchis are briefly discussed below, and Table 3 shows the standardized recipes for these three.

1. Baechu Kimchi

The subingredients used for kimchi stuffing may vary depending on family recipes and the regions where it is made. Cho et al. (13,14) standardized the recipe for baechu kimchi processing based on cookbooks, scientific papers, and publications on kimchi from manufacturing factories, and based also on chemical and functional properties. The recommended percentages of ingredients is as follows for salted cabbages (100): radish (13.0), green onion (2.0), red pepper powder (3.5), garlic (1.4), ginger (0.6), anchovy juice (2.2), and sugar (1.0) in a final salt concentration of 2.5. Fermented shrimp is not included in the authors' recipe, though the use of fermented shrimp in kimchi is popular in the Seoul area and inland regions. The use of combined fermented shrimp and fermented anchovy juice is common (68 percent) in commercial kimchi factories (2).

Table 3 Standardized Recipes of Major Kimchi

Raw ingredients	Weight ratio (%)		
	Baechu kimchi	Kaktugi	Donchimi
Korean baechu cabbage	100	—	—
Radish	13	100	100
Green onion	2.0	5.1	3.3
Red pepper powder	3.5	3.9	
Garlic	1.4	2.3	1.0
Ginger	0.6	1.0	0.3
Fermented anchovy juice	2.2	—	—
Fermented shrimp and juice	—	4.1	—
Sugar	1.0	1.4	—
Fermented green pepper	—	—	3.3

2. Kaktugi

Kaktugi is fermented radish cubes mixed with various subingredients. Our laboratories have published a standardized recipe and processing method for kaktugi based on our experiments (15) and data from the literature. The recommended ingredient combination for kaktugi are the following for salted, cubed ($2 \times 2 \times 2$ cm) radishes (100): red pepper powder (3.9), fermented shrimp (4.1), garlic (2.3), ginger (1.0), green onion (5.1), and sugar (1.4) in a final salt concentration of 2.5 percent. Three different salt processing methods are used for the cubed radishes: pickled with dry salt, pickled with brine, and non-pickled. The pickled, brined radishes, in which kaktugi is prepared after salting the radish cubes with 7 percent brine for 30 min at 5°C, showed better flavor and texture. A draining time of 1 hour after brining works well. The salted radish cubes are then mixed with red pepper powder to a it give bright red color; then there is blending with the premixtures of subingredients. Radish mixture is then placed in a vessel for fermentation (15).

3. Dongchimi

Dongchimi, a whole oriental radish (12–15 × 7–10 cm) kimchi, requires a large quantity of seasoned water. Whole radishes are salted with dry salt and then rinsed. The rinsed uncut radishes are mixed with other ingredients (shown in Table 3) in a large quantity of 3 to 4 percent brine solution and then fermented in a crock, completely immersed in the mixed ingredients and water. Green onion, garlic, and ginger are the main subingredients for dongchimi preparation. However, pears, fermented green peppers, and glue plant are frequently used. The crunchy taste of oriental pear, with its high saccharinity, and radishes combine to make the unique flavor of dongchimi when stored under 10°C for 20 days. When serving, remove the radishes from the container and slice them. Because dongchimi liquid is highly salty for storage, add water according to one's taste. The salt content of dongchimi is somewhat more than 4 percent (6).

V. FERMENTATION OF KIMCHI

A. Microorganisms and the Characteristics of the Fermentation

The fermentation of kimchi is carried out through a naturally controlled brining process. During kimchi fermentation, microorganisms should be tolerant to salt, acidity,

anaerobic conditions, and endogenous antimicrobial compounds in the ingredients. The main microorganisms involved in kimchi fermentation are LABs, which are facultative anaerobes, microaerobes, or anaerobes. Several factors, such as salt concentration, temperature, pH, microorganism population, and air exposure control the kimchi fermentation process.

The microbiological sequence of lactic acid fermentation in kimchi is similar to that of the lactic acid fermentation of sauerkraut, but it is different owing to the other subingredients, as shown before. The brining process extracts the water from the raw materials by osmotic activity and suppresses the growth of undesirable bacteria that could spoil the kimchi ingredients. At the same time, the brining conditions offer a relatively favorable environment for LAB under increased salinity.

The number of total viable bacteria, yeasts, and molds is found to be markedly decreased by 11 to 16-fold for bacteria and 29 to 87-fold for yeasts and molds in varieties of baechu cabbages imbued with 10 percent NaCl for 10 hours. In contrast, LAB levels increased to 3- to 4-fold, indicating that the brining process removes the aerobes, yeasts, and molds but stimulates growth of LAB in cabbages (10). Kim et al. (9) also reported that counts of bacteria, molds, and yeasts decreased by 45%, 58%, and 40%, respectively, by the process of salting and washing, showing that fermentation is mainly carried out by cabbage LAB after the brining process. Other microorganisms of ingredients other than the cabbages may also be involved in the fermentation, but LAB from the brined cabbage seems to be the main microorganism (12).

For the proper fermentation of kimchi, it is important to keep anaerobic conditions correct to minimize the growth of aerobic microorganisms and stimulate the growth of LAB during the fermentation. The Korean traditional earthenware crocks (Fig. 2) are excellent fermentation vessels that provide facultative anaerobic conditions during fermentation. Figure 3 shows typical microbial changes in kimchi during the fermentation at 2 to 7°C. The number of anaerobic bacteria, usually LABs, increases, whereas aerobes, such as *Achromobacter, Flavobacterium*, and *Pseudomonas* species (16) usually decrease owing to the absence of air and salt content and acid formed during fermentation. Although the level of yeast is low, film-forming yeast increases in the later fermentation stages. The yeasts isolated from the kimchi were *Saccharomyces*, *Tolulopsis*, *Debaryomyces*, *Pichia*, *Rhodotorula*, *Endomycopsis*, *Kluyveromyces*, *Cryptococcus*, *Trichospora,* and others (17).

Kimchi fermentation is initiated by *Leu. mesenteroides* (*Leuconostoc* sp.), a heterofermentative LAB and a facultative anaerobe; it produces lactic acid, acetic acid, CO_2, and ethanol as major end products. As the pH drops to 4.6 to 4.9 because of organic acid accumulation, *Leu. mesenteroides* is relatively inhibited. As shown in Fig. 4, *Streptococcus* (*St. faecalis*) behaves similarly as *Leuconostoc* sp., but in lower numbers. The fermentation continues with more acid-tolerant LAB species such as *Pediococcus cerevisiae*, *Lactobacillus brevis*, *Lac. fermentum*, and *Lac. plantarum* (18). However, there is overlapping growth of the species. Also, the growth of each species depends on its initial numbers in the cabbage and other ingredients, the concentration of salt and sugar, the absence of oxygen, and the fermentation temperature, as already mentioned. *Lac. plantarum* is present in the greatest numbers following the initial fermentation and produces the maximum acidity at the later stages, especially at higher temperatures. *Lac. plantarum* is believed to be the main acidifying or deteriorating microorganism in kimchi fermentation (19).

The presence of yeast in the later stage of kimchi fermentation can produce various tissue-softening enzymes, including polygalacturonase, which destroy pectic substances and other tissue structures of cabbages and radishes that will downgrade the kimchi quality. Softening of baechu's texture is a problem due to excessive acidification of kimchi during the overripening stage of the fermentation and preservation processes (20).

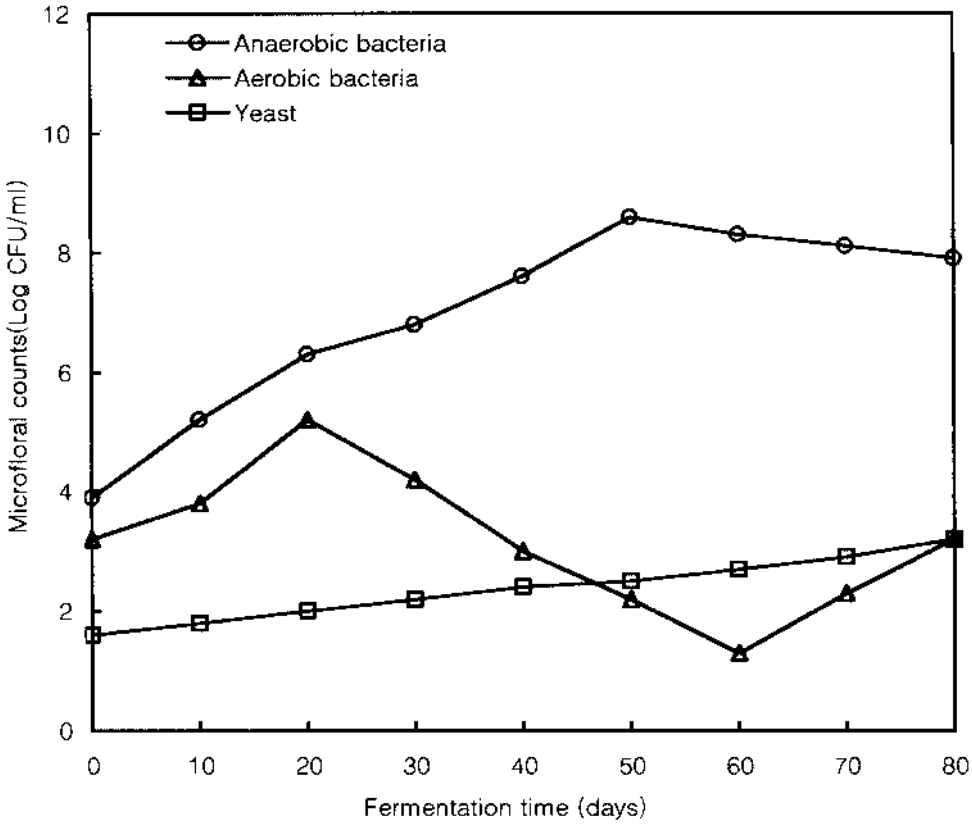

Figure 3 Microfloral changes in kimchi during fermentation at 2 to 7°C. (From Ref. 17.)

Table 4 shows the frequency of gram-positive bacteria, mainly LAB isolated from kimchi fermentation at 5, 15, and 25°C, while *Leu. mesenteroides* and *Leu. paramesenteroides* dominate (65.2%) at the low temperature of 5°C. However, the level was 13.5% at 25. The *Lactobacillus* species, mainly *Lac. plantarum*, produce lactic acid (homofermentative LAB) and dominates (59.7%) at high the temperature of 25 °C, dropping to 28% at 5°C (21). The levels of *Streptococcus* and *Pediococcus* species were lower than those of *Leuconostoc* and *Lactobacillus* species and were found to decrease considerably at lower temperatures. This suggests that a fermentation temperature of 5°C creates better conditions for producing more flavorful kimchi and extends the preservation period, mainly owing to the growth of *Leuconostoc*.

The kimchi fermentation process undergoes several distinct phases based on the changes in pH and acidity, CO_2 levels, and sugar content, all of which are temperature dependent. The first stage has a rapid decrease of pH and an increase of acidity and CO_2 levels. These changes are accompanied by a decrease of reducing sugars after the initial lag phase. The next stage shows a gradual drop in pH, a further increase in acidity and CO_2 levels, and a rapid disappearance of reducing sugars. The final stage of fermentation proceeds with no or only slight changes in pH, acidity, CO_2, and reducing sugars (3). The pH and acidity of optimally fermented kimchi are 4.2 to 4.5 and 0.4 to 0.8% as lactic acid, respectively.

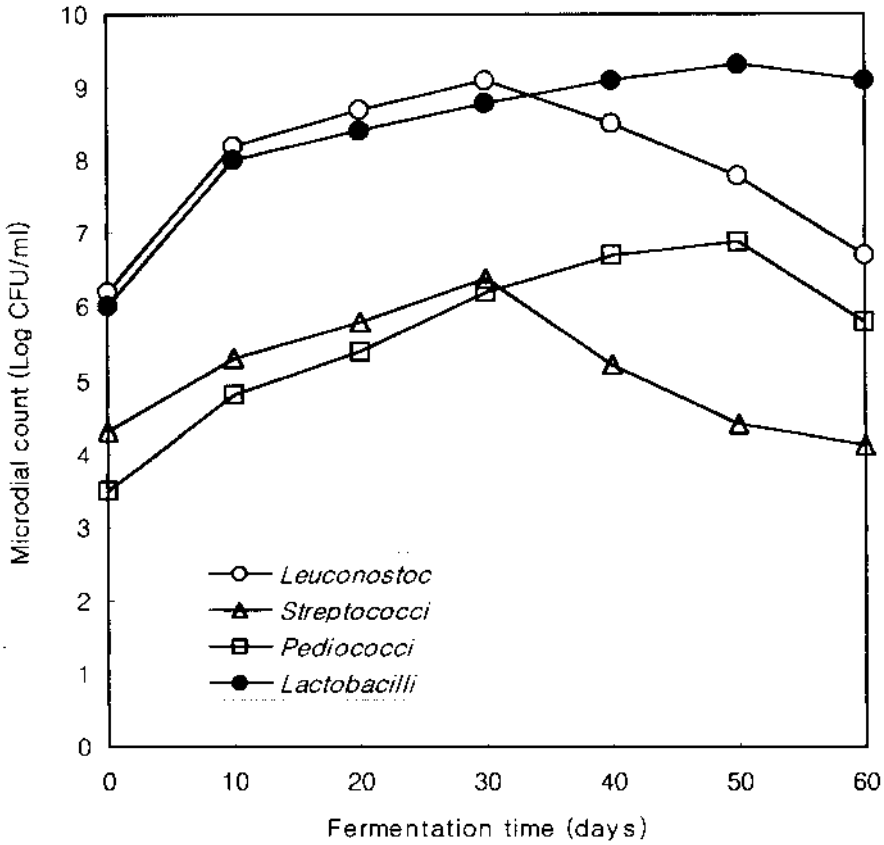

Figure 4 Microfloral changes of lactic acid bacteria during kimchi fermentation at 5°C. (From Ref. 18.)

By monitoring various fermentation temperatures, Shin et al. (22) studied the chemical changes, LAB, and yeast counts in kimchi prepared by a large-scale commercial manufacturer. The optimum pH of kimchi, around 4.2, was reached within 2 days at 25°C, 3 days at 15°C, and 23 days at 5°C. As shown in Fig. 5, the pH significantly decreased to 3.6 and stayed the same at 25°C. However, at 25°C, the pH decreased slowly to 4.1 and stayed at the same pH at 5°C. The acidity levels were opposite to the levels of pH at the different temperatures. The acidity increased up to 0.9% at 25°C after 8 days, but increased to 0.5% after 44 days, maintaining almost the same level for 80 days at 5°C. Thus a low temperature of 5°C fermentation produces the best flavor, microorganism status, and preservation conditions.

The salt content of kimchi is another important factor influencing the growth of microorganisms during fermentation. The optimum period of fermentation or the completion of the fermentation is shown in Table 5 (19). For baechu kimchi, at 3.5% salt concentration, fermentation takes 1 to 2 days and 2 days with a 5% salt concentration at 30°C. On the other hand, at the low temperature of 14°C, 5 to 12 days are required at a 3.5% salt concentration, and 10 to 18 days at a 5.0% salt concentration. If the salt content is too high at low temperature, the psychrotrophic LAB, the main microorganisms for fermenta-

Table 4 Frequency (%) of Gram-Positive Bacteria Isolated from Kimchi Fermented at 5°C, 15°C, and 25°C

Genus	Species	Subspecies	5°C	15°C	25°C
Leuconostoc	*mesenteroides*	*mesenteroides*	31.5	12	6.3
	mesenteroides	*cremoris*	0	4	4.8
	mesenteroides	*dextranicum*	10.1	0	1.6
	paramesenteroides		23.6	3	0.8
	lactis		0	1	0
Streptococcus	*lactis*		4.5	0	1.6
	iniac		0	0	0.8
	agalactiae		0	0	0.8
	raffinolactis		0	0	11.1
Pediococcus	*pentosaccus*		0	0	4.0
	inopinatus		2.2	6	0
	acidilactici		0	1	0
Lactobacillus	*plantarum*		0	15	36.5
	maltaromicus		12.3	8	5.6
	homochiochii		0	7	4.8
	brevis		0	0	3.2
	curvatus		0	0	2.4
	minor		10.1	3	0.8
	sake		4.5	9	0.8
	confuses		0	0	0.8
	hilgardii		0	0	0.8
	fructosus		0	15	0.8
	farciminis		0	3	1.6
	coryniformis	*coryniformis*	0	0	0.8
	casei	*rhamnosus*	0	0	0.8
	divergens		1.1	0	0
	alimentarius		0	4	0
	bavaricus		0	2	0
	yamanashiensis		0	4	0
	amylophilus		0	1	0
Bacillus	*cereus group*[a]		0	0	4.8
	circulans		0	0	4.0

[a] *B. cereus, B. anthracis, B. mycoides, B. thuringiensis.*
Source: Ref. 21.

tion, will not grow. Thus kimchi should be prepared at lower temperatures and lower salt concentrations and for longer times.

B. Safety of Kimchi and Bacteriocin Production

Kimchi has been eaten for centuries without causing any health problems. However, residual agricultural chemicals, pathogenic microorganisms, and NO_2 levels in cabbages are public concerns. One study reported that agricultural chemicals such as an insecticide, chloropyrifos(*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)-phosphorothioate), can be removed during the process of manufacturing kimchi (23). Baechu cabbages that were soaked in the chlorpyrifos solution were used for kimchi preparation. The residual

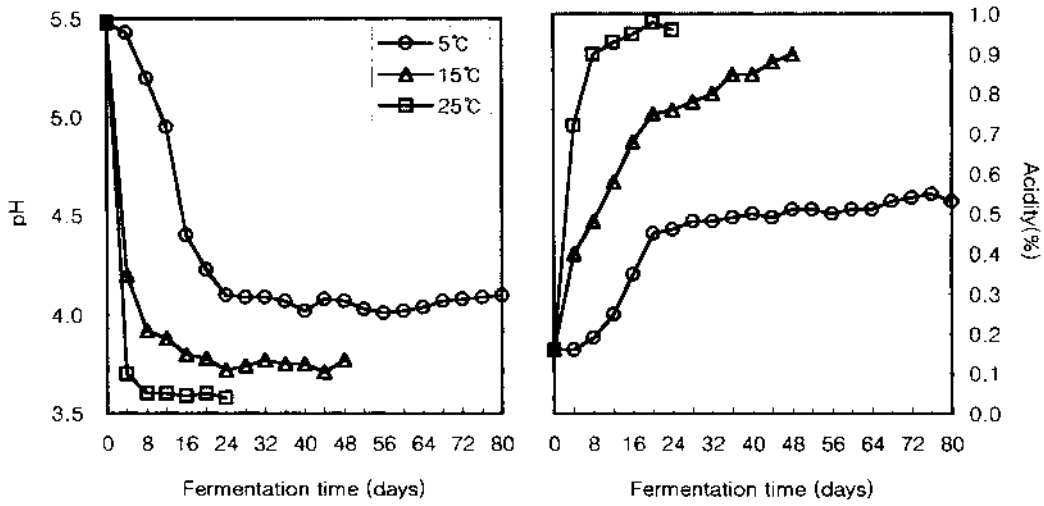

Figure 5 pH and acidity changes of kimchi during fermentation at different temperatures. (From Ref. 22.)

chlorpyrifos at 0.161 ppm in raw cabbages, decreased to 0.0938 ppm after 4 washings and then further decreased to 0.0099 ppm during the four-week fermentation.

Pathogenic microorganisms of the kimchi ingredients can be eliminated during fermentation. Park (24) examined the pathogenic microorganisms of factory-manufactured kimchi during storage at 0°C and 8°C for 41 days. The author could not detect *E. coli*, *Staph. aureus,* or *Vibrio parahaemolyticus* during the whole storage period; although, low levels of coliform bacteria were detected in the early stage of storage. The coliform bacteria level was 2.0×10^3 CFU/mL at 0 day but reduced to less than 10 count after 13 days at 8°C. The reduction of these microorganisms is likely due to the acidic and salt conditions and microbial competition during storage.

LAB in kimchi are known to inhibit the growth of pathogenic organisms. For example, *Ped. cervisiae* and *Leuconostoc* sp. are most effective in restricting the growth of pathogenic organisms, such as *E. coli*, *Staph. aureus,* and *Bacillus cereus* (25). *Ped. cervisiae* suppressed the growth of *E. coli*, *Strep. faecalis*, and *Lac. bulgaricus* (26). Using in vitro testing, the addition of ether extract from *Lac. plantarum* Lp2 isolated from kimchi culture

Table 5 Time Required for Optimum Kimchi Fermentation at Different Salt Concentrations and Temperatures (days)

	Salt concentration (%)			
Temperature (°C)	2.25	3.5	5.0	7.0
30	1–2	1–2	2	2
20	2–3	2–3	3–5	10–16
14	5–10	5–12	10–18	12–32
5	35–180	55–180	90–180	No ripening

Source: Ref. 19.

broth completely inhibited the growth of *E. coli*, *Pseud. aeruginosa,* and psychrotrophic PC1 (27).

Cho et al. (28) reported that *Lac. brevis* was found to produce an antimicrobial substance (with a molecular size of 59 kDa). It showed a wide spectrum of inhibition against gram (+) and (−) bacteria and maintained the inhibitory activity between pH 4.0 and 9.0. Paik et al. (29) isolated *Lactococcus lactis* BH5 from kimchi and identified it as a bacteriocin producer with bactericidal activity against *Micrococcus flavus* ATCC 10240. It also showed a broad spectrum of activity against most of the nonpathogenic and pathogenic microorganisms as revealed by a modified deferred method and the spot-on-lawn method. It was found that lacticin BH5 (bacteriocin produced by *Lac. lactis* BH 5, MW 3.7 kDa) is stable over a pH range of 2.0 to 9.0 and can withstand several organic solvents tested.

Baechu cabbage contains high levels of NO_3, 157–2500 ppm (30,31). One might suspect that NO_3 converts to NO_2, and then NO_2 reacts with secondary amines from fermented fishes in kimchi to form nitrosamines, which are carcinogens. Several studies have addressed the question of the safety of kimchi. Park and Cheigh (31) reviewed the subject of kimchi and nitrosamines and concluded that the level of NO_3 reduces greatly during kimchi fermentation, and that the NO_2 content was only at trace amounts. Although *N*-nitrosodimethylamine (NDMA) can be a major nitrosamine found in kimchi, the levels were trace or not detected. Interestingly, vitamin C and other antioxidative compounds in kimchi and LAB were found to inhibit NO_2 and nitrosamine formations (31,32).

C. Kimchi Fermentation with Starter Cultures

In the hope of better quality control, there have been several attempts to ferment kimchi using starter cultures. *Lac. plantarum*, *Lac. brevis*, *Ped. cerevisiae,* and *Leu. mesenteroides* isolated from kimchi were used as starters (33). The starter cultures inoculated into kimchi increased fermentation rates compared to the control kimchi, even though the fermentation period was shortened by about 24 hours compared to the control at 25°C. Also, these studies showed that the sensory test score was greater than for noninoculated control for odor, flavor, and overall acceptability.

So et al. (34) used five strains of psychrotrophic LAB from kimchi as fermentation starters. Starter-inoculated kimchi showed sharp decreases in gram (−) and coliforms from the initial stage, but the control kimchi showed slow increases in those bacteria in the early stage and then sharp decreases after 10 days at 8°C. It took 10 days to reach the optimum ripened state in the control kimchi, but only 4 to 6 days in the *Leuconostoc*-inoculated kimchi and 2 days in the *Lactobacillus*-inoculated kimchi to reach the stationary phase of the growth. Moreover, the inoculations of all these starters did not cause overacidification. The quality of *Leuconostoc*-inoculated kimchi was found to be good compared to the control but not that of the *Lactobacillus*-inoculated kimchi.

Blanching of cabbages along with LAB inoculation to kimchi was employed in another trial (35). In this study, blanching treatment before the inoculation of the starter culture reduced number of the viable cells found in the raw materials. The pH of *Leu. mesenteroides*–added group rapidly decreased but stayed steady. Blanching and the inoculation with *Leu. mesenteroides* produced kimchi with good sensory acceptability, but the speed of fermentation in the *Bifidobacterium bifidum* groups was much retarded.

Ozone water or ozone gas treatment was adopted to control microorganisms in the raw materials (36). The survival ratio of total microflora was 6 to 20 percent by treating the kimchi seasoning mixtures with ozone gas and ozone water at 6 mg/L/s for 1 hour. More than 80 percent of total viable microflora in kimchi ingredients can be removed by the ozone

treatment. Following the treatment, *Lac. acidophilus* was used as a starter culture, because it can survive in the human intestine. The ozone-treated kimchi with *Lac. acidophilus* stimulates the production of vitamin B_1 and vitamin C. The aerobic bacteria levels were greatly decreased, but the number of LAB significantly increased compared to the control. *Lac. acidophilus* produced β-galactosidase and polygalacturonase, which consumed polysaccharides in the cell wall of the cabbages, causing overripening and softening of the kimchi. Another LAB test, using a kimchi ozone sterilization combination, needs to be developed for the further study.

Single and mixed cultures of *Leu. mesenteroides* subsp. *mesenteroides* KFRI 819, *L. plantarum* KFRI 813, and *L. maltaromicus* KFRI 235 as starters for kimchi fermentation in garlic paste or skim milk as cryoprotectants at 4 and 10°C also were studied (37). The authors reported that the mixed microbial starters in 10% garlic paste showed a better quality than those in 10% skim milk, inoculated with the single culture. Since most of the raw materials used for making kimchi are heat-sensitive, new technologies must be developed to reduce initial microbial loads in the raw materials, and further research is needed on the appropriate starter cultures used in kimchi fermentation.

D. Controlled Fermentations of Kimchi to Extend Shelf Life

1. Temperature and Salt Content

Low-temperature and mild freezing storage of kimchi appear to be good choices for preservation over long periods. Table 6 shows the changes in total acidity of kimchi stored at −5 to 4°C (38). Kimchi stored at −5 to 0°C maintained 0.57–0.60% total acidity for 3 months, compared to 4°C for about 20 days. This indicates that the optimum temperature for the cold storage of kimchi is 0°C. Kimchi is traditionally prepared in early winter and stored for 3 to 4 months in pot buried underground during the winter season. One of the characteristics of the kimjang kimchi is ripening (i.e., fermentation) at low temperatures, using psychrophilic *Leu. mesenteroides*. To obtain the traditional and desirable taste of kimchi, a stepwise temperature lowering fermentation was explored. For instance, the temperature was lowered to 0°C, after a 48-hour fermentation at 20°C or for 72 hours at 15°C with 0.5% total acidity (39). Thus, at present, most Korean households use a specifically designed (i.e., temperature-programmed) refrigerator for kimchi, instead of the traditional ground-buried techniques.

There are many other ways to preserve kimchi, such as pasteurization, with and without preservatives, canning and bottling, utilization of food additives, and irradiation. Thermal processing is mainly designed to control *Lactobacillus* and other microorganisms that are responsible for overripening in the later stage of fermentation. The D-value and Z-value

Table 6 Changes in Titratable Acidity of Cabbage Kimchi During Low-Temperature Storage

Storage temperature (°C)	Storage period (days)									
	10	20	30	40	50	60	70	80	90	100
−5	0.57	0.58	0.59	0.58	0.58	0.59	0.59	0.60	0.60	0.62
0	0.58	0.61	0.60	0.61	0.59	0.60	0.59	0.60	0.61	0.62
4	0.61	0.61	0.64	0.68	0.68	0.70	0.69	0.70	0.72	0.72

Source: Ref. 38.

of related microorganisms in addition to thermal diffusability of kimchi in a retort pouch were studied (40). The canning process of kimchi was developed for several commercial products. However, the freshness of kimchi texture was totally lost owing to heat treatment. The thermal processing of kimchi has been used for the commercial distribution. This process was to destroy *Lactobacillus plantarum* or *Lactobacillus brevis*, which grow during the ripening of kimchi.

High levels of salt are another measure used to extend shelf life of kimchi. As mentioned earlier, fermentation speed is significantly delayed with increased levels of NaCl. Park and Kim (41) report that as NaCl concentration increases, CO_2 production and pH decrease significantly. Various kinds of salt manufactured in Korea affect fermentation differently; for example, heat-treated salts (guwoon salt, bamboo salt) were shown to retard the fermentation speed. Natural salt + KCl also was shown to extend the fermentation time without taste changes (42). Kim et al. (43) report that CA-A (NaH_2PO_4, Na_2HPO_4, Na_3PO_4) shortened fermentation time. Sodium phosphate and sodium citrate significantly inhibited the fermentation, while potassium phosphate had little effect. Thus the regulation of temperatures and salt levels, and their kinds and mixing techniques, are important factors for the preservation time. Therefore, a kimchi refrigerator can be used in the households that need to prepare and preserve it for long periods. The kimchi industry uses low temperature circulation. Keeping it at a low temperature is a convenient, widely used, and effective method of prolonging its shelf life.

2. Subingredients and Natural Preservative Plants

a. Subingredients. Kimchi subingredients were examined for their ability to inhibit the growth of microorganisms. An increased garlic concentration (0–6%) in the preparation decreased the number of aerobic bacteria but increased LAB levels at 2 days (early stage fermentation) at 21°C (44). A recipe of 2 percent garlic content in kimchi decreased the amount of aerobic bacteria significantly (50- to 1000-fold) compared to a kimchi recipe of 1% garlic. Cho and Jhon (45) also reported that 21 aerobic bacteria isolated from kimchi had inhibited growth in the presence of garlic extract. The isolated bacteria were 11 *Bacillus* sp., 2 *Staphylococcus* sp., 1 *Micrococcus* sp., 1 *Flavobacterium* sp., 2 *Enterobacteriaceae* and 4 *Vibrionaceae*. The 21 aerobic bacteria could not grow in the nutrient broth with a 4.5% garlic concentration at 30°C for 24 hours. Only one strain survived when the garlic concentration was 2.8%. Nine out of the 21 strains could survive in the 1% garlic concentration. Kim et al. (46) reported that garlic extract also significantly suppressed the growth of *E. coli* in tryptic soy broth (TBS). Based on the data, these investigators concluded that garlic is a major condiment having the ability to eliminate unnecessary microorganisms of aerobic bacteria and *E. coli* in kimchi.

Lee and Kim (47) reported that red pepper, garlic, and ginger (the main subingredients of kimchi) also inhibited growth of the LAB at only the early stage of fermentation (2 days at 25°C), but thereafter the overall growth rate was the same or accelerated compared to the control.

On the other hand, sucrose, MSG, fresh oyster, and salt-fermented anchovy and shrimp increased the fermentation rate (48). MSG reduced kimchi the fermentation period, by the stimulatory growth of the LAB, but the pH changes were similar to the control (49). The addition of MSG to kimchi showed growth stimulation of LAB with a buffering action and a constant pH level. But MSG affected the stability of ascorbic acid, thiamine, and β-carotene. The MSG addition enhanced the flavor because of glutamic acid, which reduced the sour taste.

b. Natural Preservatives. To develop a natural preservative that would extend the shelf life of kimchi, Moon et al. (50) screened 102 edible plants, 21 antimicrobial agents, and other related compounds for kimchi fermentation. *Baical skullcap* and *Assam indigo* from 42 oriental medicinal plants were shown to be effective for maintaining the fresh state of kimchi. Thirty-two herbs and spices, including peppermint, cinnamon, lemon balm, clove, hops, rosemary, sage, horseradish, and thyme showed high antimicrobial activity against the kimchi-fermenting microorganisms. Pine needles, persimmons, and oak leaves showed a significant bactericidal effect among the 28 fruits, vegetables, and related plants tested. Nisin and caffeic acid effectively slowed kimchi fermentation, making them good natural preservatives in extending the shelf life of kimchi. However, sensory evaluation is another important factor to consider for the selection of preservatives. Kim and Park (51) studied antimicrobial activities among 15 kinds of vegetables used as kimchi ingredients. Leek extracts showed particularly strong antimicrobial activities against *Ped. cerevisiae* and *Lac. plantarum,* known to be a major microorganism for the acidification of kimchi during fermentation.

Saps from pine needles are reported to suppress fermentation (52), as the pine needles extend by almost double the time needed to reach a pH 4.3. The total viable number and the *Lactobacillus* cell number decreased when pine needle extract was added to kimchi, regardless of fermentation temperatures.

The addition of green tea in kimchi extended its shelf life by lowering the titratable acidity, lactic acid, and acetic acid contents (53). We studied the increased preservative and antimutagenic effects of green tea leaves added to baechu kimchi (green tea added kimchi, GK) (54). The fermentation period for the GKs was extended compared to the control kimchi (CK). Although the initial pH and acidity between GKs and CK were similar, the time for the kimchis to reach optimally ripened status (pH 4.3) was different. CK took 6 days, while GK2 and GK4 took 10 and 14 days at 10°C, respectively (Fig. 6). Accordingly, the growth of *Leuconostoc* sp. and *Lactobacillus* sp. in the GKs was considerably delayed. As shown in Fig. 7, the growth of *Leuconostoc* sp. was greatly retarded, yet the levels are still

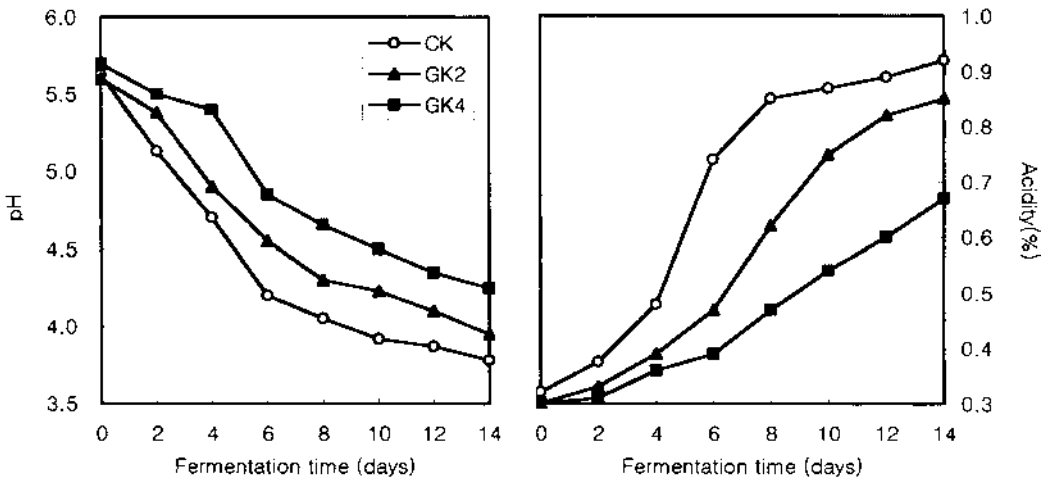

Figure 6 Changes in pH and acidity of control kimchi (CK) and green-tea-leaves added kimchis (GK) during fermentation at 10°C. GK2: 2% green-tea-leaves-added; GK4: 4% green-tea-leaves-added. (From Ref. 54.)

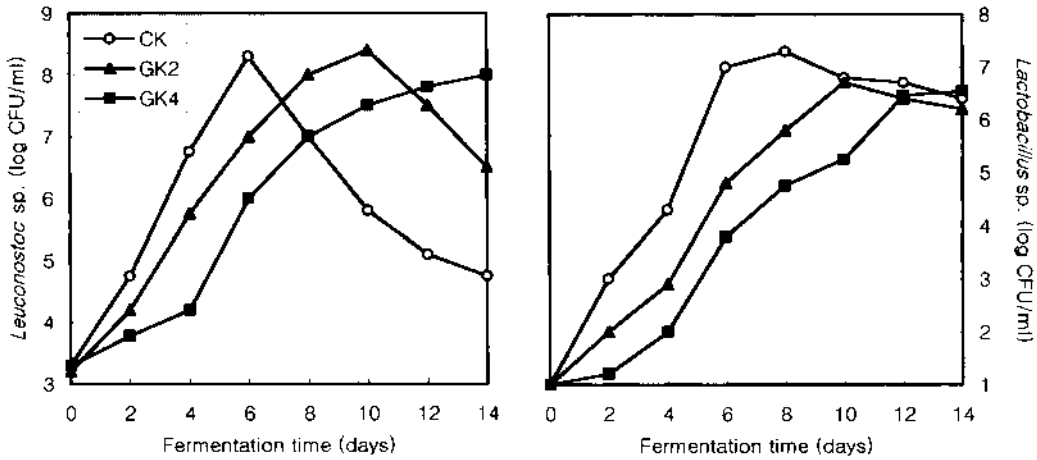

Figure 7 Changes in the numbers of *Leuconostoc* sp. and *Lactobacillus* sp. of control kimchi (CK) and green-tea-leaves-added kimchi (GK) during fermentation at 10°C. GK2: 2% green-tea-leaves-added; GK4: 4% green-tea-leaves-added. (From Ref. 54.)

high compared to the control. However, the population of *Lactobacillus* decreased with the addition of green tea, indicating that *Leuconostoc* sp. was dominated to *Lactobacillus,* giving a better taste to the kimchi.

The addition of ginseng (2%) to the kimchi boosted both its shelf life and its quality. Ginseng-added kimchi showed a more favorable tissue texture, improved overall acceptability, and retarded the rancidity of the kimchi (55,56).

The effects of low temperature heating and the addition of mustard oil on pH and total acidity of kimchi during storage at 15°C were studied (57). Mustard oil showed antimicrobial acitivity for *Lac. plantarum*, *Lac. brevis*, *Leu. mesenteroides,* and *Ped. cervisiae*. The addition of 200 ppm mustard oil, 0.1% mustard powder, and 0.01% H_2O_2 to kimchi reduced the fermentation rate. Low temperature heating (50°C) of salted cabbages and the addition of 200 ppm mustard oil and 0.01% H_2O_2 to seasonings extended the time to reach the optimum ripening of the kimchi, about 2.5 times compared to the control. A combination of preheating at a low temperature (50°C for 35 minutes) with addition of mustard oil and H_2O_2 to the seasoning and postheating at low temperature (65°C for 20 minutes) slowed the kimchi fermentation speed about 5 times, compared to the control.

Chitosan and oligochitosan decreased the fermentation rate. The pH was not lowered, and total acidity was lower, in the chitosan-added kimchi (58). The numbers of total viable cells, *Leuconostoc* sp. and *Lac. plantarum,* were lower in the chitosan-added kimchi than in the control. The sour and stale flavor of the kimchi was reduced by the chitosan addition. Our laboratories found that when the chitosan oligosaccharide is added (1%), the growth of LAB can greatly be inhibited, without a decrease of pH. The increase in total acidity was greatly retarded (more than twice) during fermentation at 15°C. More importantly, the taste of the final product received a high mark by the sensory evaluation test.

Chinese pepper also showed an antimicrobial property to extend the shelf life of kimchi. It is worth noting that experiments show that peppers have antimutagnic/anticancer activities in tests in vitro and in vivo (59). Choi et al. (60) studied the inhibitory effect of nisin (a bacteriocin produced by *Strep. lactis*) on kimchi fermentation at 15°C. The addition of 100 IU/g of nisin delayed fermentation by pH, total acidity, and LAB counts.

Bamboo leaves extract exhibited strong antimicrobial activity against *Brettanomyces custersii*, *Klebsiella oxytoca,* and *Pichia membranaefaciens*, which are responsible for softening the kimchi texture (61). The antimicrobial activity of bamboo leaves extract was better than 0.5% and 1.0% sorbic acid, and was stronger at pH 5 than at pH 7. The addition of bamboo leaves to radish-based dongchimi is an old tradition in prolonging the shelf life and improving the taste of kimchi (62).

3. Increased Shelf Life of Kimchi by Fermentable Sugar Reduction

Kim et al. (63) studied the sugar content of radishes to improve the storage stability of cubed radishes by monitoring the titratable acidity (TA) at the completion of fermentation. The final TA obtained at the end of fermentation was directly proportional to the sugar content of the radishes, with a value of $0.29x + 0.4428$, where x is the sugar content of the radish. These investigators also studied the relation between the soluble solids (SS) content of baechu cabbage and the final TA value of the kimchi (64). The results showed that the final TA in the kimchis studied was also directly proportional to the SS content (x) of the cabbage, showing that TA is equal to $0.3x + 0.7779$.

Yu et al. (65) also studied the extension of the shelf life of kimchi by reduction of the initial reducing of sugar content (s_0). With initial sugar content reductions of 2.3%, 0.97%, and 0.6%, fermentation periods to produce 0.75% TA required 2, 7, and 12 days, respectively. Thus these researchers were able to estimate the fermentation period (T) to produce 0.75% acid in kimchi using the equation $T = -16.82 \log(s_0) + 7.66$. Kimchi with baechu cabbage devoid of about 80% of fermentable sugar can extend a storage period by 30 days at 25°C with 0.8% total acidity, but the score of sensory evaluation was low.

The reducing sugar content (5.7mg/mL) of baechu cabbage using a salting and desalting process prior to kimchi fermentation took 6 days to reach 0.75% total acidity, but the control sample containing 15.1 mg/mL of sugar took 3 days of fermentation to reach the same level of total acidity, 0.75%. The hardness value of kimchi texture of the treatment was higher than that of the control during the fermentation period (66).

4. Preservation and Packaging During Marketing

Low temperature by refrigeration or mild freezing is a preferred preserving method during storage for marketing purposes. As already shown, kimchi stored at 4°C maintained a high quality for only 20 days, while kimchi stored at −5°C to 0°C keeps its quality for up to 3 months (38). The salinity of kimchi lowers the freezing point, but the texture of kimchi cabbage is not affected as low as −5°C even though the kimchi broth is frozen. Thus it is recommended that kimchi be fermented at a low temperature range (7 to 15°C) and stored at a lower temperature around −1°C (67). For storage longer than 4 months, quick freezing is recommended to prevent texture degradation. The texture of kimchi that is frozen at −15°C is damaged significantly after freezing and thawing, although little change in taste and flavor takes place. If kimchi products are packaged, and quickly placed in brine at −30°C to −38°C, and remain in a freezer, the texture will be well maintained (68).

In our experience, vacuum packaged bottled or canned kimchi had a longer shelf life than the control. Lee et al. (69) reported that zeolite sealed in 10 μm thick, high-density polyethylene film could considerably reduce pressure and volume increase by CO_2 production when included inside the kimchi packages at 15°C.

Kim et al. (70) evaluated the quality of kimchi affected by the packaging materials Ny/PE, Ny/CPP, Cryovac BK−1, BK−4, and PET/Al/PE films during storage at 5°C and 20°C. The CO_2 concentration of kimchi packaged with cryovac BK-1 and BK-4, which has higher gas permeability, increased to a maximum and then decreased owing to gas per-

meation during storage. It should be mentioned that LAB levels and the sensory evaluation of these products were not significantly different. Thus it is recommended that CO_2 permeable films be used rather than high gas-impermeable barrier films to avoid swelling of packaged kimchi during storage and distribution.

The Miraflash (71) plastic container (polyethylene + natural antimicrobial materials) was studied in comparison with polyethylene and aluminum film kimchi packaging characteristics. The Miraflash container was developed based the rationale that the synthetic materials may mimic the traditional Korean porous potteries. In addition, Miraflash has antimicrobial activity and no known environmental hormones. When kimchi is fermented in the Miraflash container, pH decreases and the total acidity increases slowed. Also, the *Lactobacillus* sp. counts were significantly decreased after the optimal ripening period. This artificial container seems to mimic traditional Korean pottery, thereby helping to extend preservation periods and preserve the traditional taste of the kimchi without the contamination of environmental hormones.

Hong and Park (72) developed color indicators on kimchi packages as an innovative way to monitor the ripeness of commercial kimchi products during storage and market distribution. In their design, Hunter b values of bromocresal purple type and Hunter a values of methyl red type appeared to be proportional to both the pH and the titratable acidity of kimchi. Kimchi was packed in a polypropylene (PP) tray with a nylon/cast polypropylene (Ny/CPP) lid, where the indicating sachet consisting of CO_2 absorbent and chemical dye (bromocresol purple and methyl red) was attached. This technique is an effective way to sense the ripeness of packaged kimchi products.

VI. BIOCHEMICAL, NUTRITIONAL, AND FUNCTIONAL PROPERTIES OF KIMCHI

A. Biochemical Changes During Kimchi Fermentation

1. Flavor Compounds

a. Organic Acids. Organic acids produced during kimchi fermentation create the main flavor of kimchi. As shown in Table 7, lactic acid content increases, but other non volatile organic acids contents are high at the beginning and decrease during fermentation (73). The lactic acid content is highest in the red pepper–added kimchi and then in garlic-added kimchis, meaning that condiments added to the baechu cabbage stimulate the lactic acid production. Lactic acid and succinic acid contents are high at lower temperatures (6 to 7°C) than at higher temperature (22 to 23°C). Acetic acid is the main volatile organic acid produced during kimchi fermentation. Garlic-added kimchi produced highest levels of acetic acid. Levels of propionic acid, butyric acid, valeric acid, caproic acid, and heptanoic acid are relatively low. Heterofermentative LAB, such as *Leuconostoc mesenteroides* and *Lactobacillus brevis,* could produce acetic acid. These findings indicate that garlic likely supports the growth of heterofermentative LAB. The CO_2 content was also high in garlic-added kimchi. Chyun and Rhee (74) reported that low salt levels and low temperature support increased production of acetic acid.

b. Volatile Flavor Components. Forty different aroma components have been identified in kimchi using the dynamic headspace concentration method (75). The major aroma components are ethanol, methyl allyl sulfide, acetic acid, dimethyl disulfide, camphene, 1-phellandrene, diallyl disulfide, methyl allyl trisulfide, e,-zingibirene, etc. These compounds increase during the ripening period and then decrease. Methyl allyl sulfide content increased

Table 7 Organic Acids Content in Various Kimchi at 12–16°C (meq/100 g)

Sample	Kimchi A			Kimchi B			Kimchi C		
Fermentation period (day)	1	4	7	1	4	7	1	4	7
Nonvolatile organic acids									
Lactic acid	0.07	0.14	0.33	0.08	0.62	0.99	0.19	0.83	1.64
Succinic acid	0.70	0.35	0.29	0.30	0.87	0.82	0.08	0.83	0.69
Fumaric acid	0.48	t	t	t	t	t	0.04	t	t
Malic acid	3.25	1.24	t	3.65	0.27	0.61	1.04	0.09	t
Volatile organic acids									
Formic acid	ND	t	ND	ND	ND	ND	ND	t	ND
Acetic acid	0.27	0.64	1.84	t	2.53	7.09	0.27	0.81	4.82
Propionic acid	0.16	0.23	0.54	1.62	1.43	0.23	1.51	1.50	1.62
Butyric acid + Valeric acid	0.51	0.76	0.82	0.38	0.54	0.41	0.44	0.76	0.68
Caproic acid	0.03	0.11	0.11	0.04	0.06	0.05	0.07	0.07	0.08
Heptanoic acid	0.04	0.11	0.11	0.03	0.04	0.05	0.05	0.26	0.08

Kimchi A: cabbage (100%); B: cabbage (100%) + garlic (4%); C: cabbage (100%) + red pepper 4 (%); t: trace, ND: Not detected.
Source: Ref. 73.

suddenly, 30 fold, and decreased gradually. The methyl allyl sulfide content correlated well by $r = 0.93$ by sensory scores. Thus the methyl allyl sulfide produced during fermentation seems to be a major volatile flavor compound in well-fermented kimchi.

c. Amino Acids Contents. The amino acids that form during fermentation are other flavor-generating sources for kimchi. Table 8 shows free amino acid contents in kimchi that was fermented at 20 to 22°C for 44 to 47 hours, with and without fermented anchovy liquid (76). Glutamic acid, arginine, lysine, aspartic acid, and alanine were the major amino acids found in well-ripened kimchi. Amino acids are affected mainly by the addition of ingredients such as salt-pickled seafoods and meats. Though microorganisms consume some amino acids during fermentation, amino acids accumulate via protein hydrolysis of the ingredients.

The total free amino acid levels greatly increased by the addition of fermented anchovy juice. The contents of lysine, aspartic acid, glutamic acid, valine, methionine, isoleucine, and leucine resulted in significant increases and serve as flavor enhancers for the kimchi (Table 8). Park et al. (2) indicated that commercial kimchi factories are using fermented anchovy juice for the production of glutamic acid and other amino acids to give a better flavor.

2. Vitamins

Kimchi is known to be a good source of vitamins in the Korean diet. The raw ingredients used for kimchi preparation contain high levels of vitamins, but some vitamins can be synthesized during the fermentation.

Table 9 shows vitamin contents of common kimchi, the average contents of vitamins in three different starter-inoculated kimchis, and the common kimchi during fermentation at 3 to 7°C (77). The levels of vitamin B_1, B_2, B_{12}, and niacin are all increased during the course of fermentation. The vitamin levels of the common kimchi without inoculation of the starters increased similar to the preparation with starter cultures. Vitamin B_1 level increased twice, but B_2 increased almost four times, and vitamin B_{12} and niacin also increased. The maximum levels of these vitamins were in the optimally ripened kimchi state of 2 or 3 weeks.

Table 8 Free Amino Acid Content in Kimchi Fermented at 20–22°C for 44 to 47 h with and without Addition of Fermented Anchovy Solution

Amino acid	Kimchi[a] without fermented anchovy solution	Kimchi with fermented anchovy solution	
		10 mL[b]	15 mL[c]
Lysine	0.21 (7.5)[d]	1.10 (14.5)	1.32 (12.0)
Histidine	0.07 (2.4)	0.01 (0.2)	0.11 (1.0)
Arginine	0.29 (10.3)	0.40 (5.3)	0.60 (5.5)
Tryptophan	0.22 (7.7)	0.12 (1.5)	0.27 (2.5)
Aspartic acid	0.17 (5.8)	0.78 (10.4)	1.20 (10.9)
Threonine	0.40 (14.0)	0.65 (8.6)	0.69 (6.3)
Serine	—	0.46 (6.1)	0.58 (5.3)
Glutamic acid	0.27 (9.7)	0.94 (12.5)	1.50 (13.7)
Proline	0.11 (3.8)	0.24 (3.2)	0.35 (3.2)
Glycine	0.07 (2.5)	0.22 (2.9)	0.34 (3.1)
Alanine	0.52 (18.4)	0.86 (11.4)	1.22 (11.2)
Valine	0.15 (5.2)	0.49 (6.5)	0.78 (7.2)
Methionine	0.02 (0.5)	0.16 (2.2)	0.26 (2.3)
Isoleucine	0.10 (3.4)	0.30 (4.0)	0.47 (4.3)
Leucine	0.10 (3.7)	0.49 (6.5)	0.76 (6.9)
Tyrosine	0.08 (2.7)	0.12 (1.6)	0.17 (1.6)
Phenylalanine	0.07 (2.5)	0.22 (2.9)	0.32 (2.9)
Total	2.83 (100)	7.57 (100)	10.94 (100)

Ingredient ratio of kimchi: 100 Chinese cabbage, 4 leek, 2 garlic, 1 ginger, 2 red pepper flour, 1 sugar.
[a] 10mL of 15% table salt solution added.
[b] 10mL of fermented anchovy solution (salt concentration 24% added).
[c] 15mL of fermented anchovy solution (salt concentration 24% added).
[d] % in total amino acid.
Source: Ref. 76.

Table 9 Vitamin Contents of Common Kimchi and Average Vitamin Contents of 4 Different Kimchi During Fermentation at 3–7°C

Fermentation time (week)	Carotene (μg %)	Vitamin B_1 (μg %)	Vitamin B_2 (μg %)	Vitamin B_{12} (μg %)	Niacin (μg %)	Vitamin C (mg%)
0	49.5[a]	41.7	66	0.17	740	28.9
1	44.0 (35.4)[b]	41.6 (40.1)	47 (54)	0.09 (0.09)	781 (747)	25.0 (25.3)
2	32.0 (30.4)	70.9 (61.9)	110 (99)	0.19 (0.20)	928 (861)	27.8 (28.5)
3	26.6 (26.9)	79.1 (87.5)	230 (157)	0.25 (0.33)	901 (792)	23.6 (22.3)
4	21.0 (25.3)	62.7 (70.8)	35 (95)	0.20 (0.26)	591 (525)	16.7 (16.0)
5	24.2 (20.1)	53.3 (49.1)	40 (37)	0.10 (0.16)	—	11.6 (11.0)

[a] Naturally fermented baechu kimchi.
[b] Average levels of four different kimchis; common kimchi + 3 different starter inoculated kimchis.
Source: Ref. 77.

Carotene levels of the kimchi gradually decreased during fermentation. The initial content of 49.5% decreased to 27% after 3 weeks. The vitamin C level slightly decreased at an early stage of fermentation but rebounded to the optimally ripened states, indicating synthesis of vitamin C during fermentation. Another experiment also showed the same result for vitamin C content. The vitamin C content was 15 mg% at the beginning but increased 17 mg% after a slight decrease (3).

3. Acidification and Overfermentation

Various biochemical changes occur during fermentation along with the formation of acceptable flavor and texture. However, undesirable fermentation or overfermentation after ripening may produce a poor-quality kimchi, resulting in acidification and cabbage tissue softening (78). Because the raw materials contain sufficient sugar to convert to organic acids, excessive acid can be formed with continuous fermentation by more acid-tolerant microorganisms. The tissue softening problem is associated with the decomposition of pectic substances in the tissues of cabbages or radishes (79). Polygalacturonase, which is responsible for tissue softening, shows a higher activity during the later fermentation period and is known to be excreted primarily from aerobic and surface-film-forming microorganisms. Also, changes in chlorophyll compounds and other substances occur during the storage of fermented kimchi.

B. Nutritional Characteristic of Kimchi Ingredients and Kimchi

1. Kimchi Ingredients

The nonfibrous carbohydrate that is consumed by endogenous LAB is high in red pepper powder and garlic, even though the main sugar source is baechu cabbage. Most subingredients for kimchi contain Ca and P. Especially high amounts are found in red pepper powder, fermented shrimp, and fermented anchovy. Carotenoids and bioflavonoids are found in baechu cabbage, green onion, carrot, and red pepper powder. Baechu cabbages and red pepper powder are the main sources of vitamin C. Ginger contains gingerol, which stimulates appetite, blood circulation, perspiration, and antimicrobial function, and can be a source of niacin and vitamin K. Oysters are a main source of vitamin B complex. Garlic is known to have various health benefits. For example, allin, one of the major active components of garlic, converts to allicin by the action of alliinase. Allicin by combining with vitamin B_1 makes allithiamine, which makes vitamin B_1 available in the body for the production of energy. It is known that allicin, S-containing compounds, and methyl linoleate in garlic exert antimutagenic and anticancer effects (80,81).

Fermented anchovy juice is source of protein, amino acids, Ca, P, and Fe. Radishes offer sugar, niacin, Ca, and isothiocyanate during fermentation. Green onion is a source of vitamins A, and C and chlorophyll. In addition, kimchi ingredients contain large amounts of phytochemicals (82).

2. Kimchi

Kimchi has a low caloric content, but it is rich in minerals, vitamins, and dietary fiber. The protein and lipid contents can be increased with various subingredients like fish, clams, oysters, and meat, which give kimchi its characteristic savor. Triglycerides, polar lipids, free fatty acids, monoglycerides, hydrocarbones, sterol, and about 18 various free fatty acids were found, among the major fatty acids (44 to 60%) are linoleic and linolenic acid. The

vitamin C and carotene content in kimchi are from the vegetables, while vitamin B complex comes from fermented fish and is synthesized during fermentation (77).

Some phytochemicals such as benzylisothiocyanate, indole compounds, thiocyanate, and sitosterol have been found in kimchi and are known to have antimicrobial and anticancer functions. The total dietary fiber (DF) content of kimchi is about 24% on a dry basis. The contents of SDF (soluble dietary fiber) and IDF (insoluble dietary fiber) are 7.8% and 16.2%, respectively (83). The nutritional composition of baechu kimchi, kaktugi, and dongchimi, the most popular kimchis, are shown in Table 10 (84). Because kimchi is made with a mixture of ingredients, the mixing ratios and the variety of the ingredients cause individual nutritional characteristics.

Although energy sources are low (11 to 18 kcal/100 g), baechu kimchi and kaktugi are especially good sources for Ca (37 to 47 mg), P (40 to 58 mg), and K (300 to 400 mg). There are 0.06 and 0.14 mg of vitamin B_1, 0.06 and 0.05 mg of vitamin B_2, 0.8 and 0.5 mg of niacin, and 14 and 19 mg of vitamin C in 100 g of baechu kimchi and kaktugi, respectively. The nutritional value of dongchimi is low as this kimchi contains large amounts of water.

Thus kimchi are low in calories, low in sugar and lipids, and high in vitamins, minerals, dietary fibers, and organic acids, especially LAB (10^7 to 10^9/g). Also, various condiments may be added to kimchi, thereby enhancing particular nutrients or specific functional ingredients.

C. Functional Properties of Kimchi

As already shown, the main ingredients of kimchi are yellow and green vegetables, which have been claimed to prevent cancer, increase immune functions, slow the aging process, and prevent constipation. In addition, kimchi augments the taste of the raw vegetables and is a good probiotic food as in yogurt.

Table 10 Nutritional Value per 100 g of Major Kimchi

Composition	Baechu kimchi	Kaktugi	Dongchimi
Energy, kcal	18	33	11
Moisture, %	90.8	88.4	94.2
Protein, g	2.0	1.6	0.7
Fat, g	0.5	0.3	0.1
Nonfibrous carbohydrate, g	2.6	6.7	2.5
Fiber, g	1.3	0.7	0.5
Ash, g	2.8	2.3	2.0
Calcium, mg	47	37	18
Phosphorus, mg	58	40	17
Iron, mg	0.8	0.4	0.2
Potassium, mg	300	400	120
Vitamin A, RE	48	38	15
β-carotene, μg	290	226	88
Vitamin B_1 mg	0.06	0.14	0.02
Vitamin B_2 mg	0.06	0.05	0.02
Niacin, mg	0.8	0.5	0.2
Vitamin C, mg	14	19	9

Source: Ref. 84.

Table 11 summarizes the functionality of kimchi. Kimchi increases the appetite owing to its flavor, texture, color, etc. The fresh taste of its vegetables, the taste from lactic acid bacteria fermentation, and the flavors of the condiments, including red pepper powder, garlic, ginger, and fermented anchovy, all contribute to kimchi's characteristic flavors. It is also known to prevent constipation and colon cancer owing to the high content of organic acids, LAB, and dietary fiber. The content of dietary fiber in kimchi is high, about 24% (83); dietary fiber plays roles in the prevention of hypertension, diabetes, constipation, and cancer.

The LAB in kimchi can be a good source of probiotics. Several cell wall components of LAB have been shown to increase the immune function and to prevent cancer (85). The intake of kimchi reduces the level of *E. coli* and increases LAB, especially *Lactobacillus* and *Leuconostoc* in the human intestines (86). The freeze dried cell bodies of LAB found in kimchi revealed strong antimutagenic activity against 4-NQO (4-nitroquinoline-1-oxide) and MeIQ (87,88). *Leu. mensenteroides*. *Lac. plantarum* and *Lac. fermentum* from kimchi showed almost same strength of antimutagenicity as *Lac. acidophilus* from dairy products. Kimchi intake significantly reduced β-glucosidase and β-glucuronidase, toxic enzymes known to transform precarcinogens into carcinogens in the human colon, and decreased pH (82). This fact is well correlated with the low incidence of colon cancer among Koreans.

Kimchi intake reduces serum cholesterol and increases fibrinolytic activity (89), and thereby shows antiatheroscleotic activity. Kimchi may retard skin aging processes (90) owing to its antioxidative properties from vitamin C, β-carotene, phenolic compounds, chlorophyll, and so forth. Kimchi also contains β-sitosterol, PUFA derivatives, glucosinolates, isothiocyanates, indoles, and allyl compounds. These compounds play various roles in the prevention of cancer and the increasing of immune function (80,82).

Since various subingredients can be added to kimchi, conceivably one can formulate a functional kimchi that provides many health-benefiting ingredients such as vitamin C-enhanced kimchi, anticancer kimchi, antiaging kimchi, etc. Certainly, kimchi can be considered as a protective food, since it contains high levels of vitamins, especially C, and minerals.

1. Control of Body Weight

Kimchi is shown to reduce body weight in rats. The capsaicin in red pepper stimulates spinal nerves and activates the release of catecholamine in the adrenal gland (91), a hormone known to increase metabolism. As shown in Table 12, when rats were fed a diet containing red pepper powder plus a high fat content, these animals had a decrease in body weight, compared to rats fed only a high fat diet (92,93). When the rats are fed kimchi that contained the same level of red pepper powder plus high fat as in the previous diet, it was found that the

Table 11 Nutritional and Health Benefits of Kimchi

1. Increase in appetite
2. Control of body weight
3. Prevention of constipation and colon cancer
4. Good source of probiotics (lactic acid bacteria)
5. Decrease in serum cholesterol, and increase in fibrinolytic activity
6. Antioxidative effect (antiaging, prevention of skin aging)
7. Anticancer effect (antimutagenic and antitumor effect)
8. Increase in immune function

Table 12 Changes in Body Weight and Food Intake of Rats Fed Experimental Diet After 4 Weeks

	Normal	High-fat diet (HFD)	HFD + 5% red pepper powder	HFD + 10% kimchi
Body weight (g)				
Initial weight	171.4 ± 11.9^{ns}	170.3 ± 10.0	170.7 ± 6.3	171.4 ± 4.1
Final weight	305.7 ± 12.7^{b}	338.7 ± 13.4^{a}	311.0 ± 9.5^{b}	302.6 ± 11.3^{b}
Food intake (g/day)	19.1 ± 0.9^{ns}	19.9 ± 0.8	19.5 ± 1.1	19.4 ± 0.9

^{a,b}Means with different letters in the same row are significantly different ($p < 0.05$) by Duncan's multiple range test.
ns: not significant.
Source: Ref. 93.

rats had a further decrease in weight after 4 weeks. Regarding individual organ weight, kimchi plus a high-fat diet significantly reduced liver weight in rats compared to rats fed the high-fat diet. The liver weight of the kimchi-fed rats was even lower than that of the non-treated group. However, the weights of spleen and kidney were not significantly different among the treatment groups (93). The epididymal fat pad and perirenal fat pad were also reduced in animals fed red pepper powder and kimchi added diets, compared to the control group, fed the high-fat diet. The body weights of the rats in the kimchi diet group significantly decreased, especially the weight of the perirenal fat pad, compared to both the red pepper powder diet group and the high-fat diet group (93). The reduction of body weight by kimchi might be due to the red pepper powder, garlic, dietary fibers, among other things.

2. Anticancer Effect of Kimchi

a. Antimutagenic/Anticarcinogenic Effect. Kimchi showed antimutagenic activities against aflatoxin B_1 in Ames test, SOS chromotest in vitro (94–96). The kimchi extract also showed antimutagenic activity in the *Drosophila* wing hair spot test in vivo (97). Kimchi also exhibited anticlastogenic activity in mitomycin C–induced mice, using the in vivo supravital staining micronucleus assay (98).

C3H/10T1/2 cells are mouse embryo cells that form foci in culture media when exposed to carcinogens. The foci, developed as type II and type III, are well correlated with tumor formations in C3H mice, 50% and 85%, respectively (99). The transformation of C3H/10T1/2 cells markedly decreased when kimchi extract (methanol soluble fraction, MSF) was added to the test system (100). When MSF (200 μg/mL) from 3-week-fermented kimchi at 5°C was added along with 3-methylcholanthrene (MCA) to the cells, then the numbers of type II and III foci formed substantially decreased from 7.4 (MCA only) to 0.8.

b. Anticancer Effect. The kimchi extracts inhibited the survival or growth of human cancer cells (AGS gastric cancer cells, HT-29 colon cancer cells, MG63 osteocarcinoma cells, HL-60 leukemia and Hep 3B liver cancer cells) in the SRB (Sulforhodamine B) assay, the MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, and the growth inhibition test (59,94). The kimchi fraction inhibited the ^{3}H thymidine incorporation in the cancer cells (101). In our studies, sarcoma 180 cells were transplanted to the Balb/c mouse followed by kimchi extract injection, and the MSF (methanol soluble fraction) of the kimchi treated group resulted in the smallest tumor weight of 1.98 ± 1.8 g compared to the control group of 4.32 ± 1.5 g (102). MSF from 3-week-fermented kimchi reduced malondialdehyde (as lipid peroxidation marker) formation, and the hepatic cytosolic

Table 13 Inhibitory Effect of Methanol Extracts from Common Kimchi and Functional Kimchi on Tumor Metastasis Induced by Colon 26-M3.1 Cells

Treatment	Dose (mg/mouse)	No. of lung metastasis (inhibition, %)	
		Mean ± SD	Range
Control		162 ± 7[a]	153–172
Common kimchi	0.05	157 ± 13[a] (3)	142–172
	0.25	147 ± 8[ab] (9)	138–157
	1.25	139 ± 5[bc] (14)	131–144
Functional kimchi	0.05	119 ± 4[d] (27)	114–123
	0.25	99 ± 8[e] (39)	89–110
	1.25	83 ± 6[f] (49)	73–91

[a–f] Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.
Source: Ref. 42.

xanthine oxidase activity in sarcoma-180 treated Balb/c mouse. On the other hand, MSF increased the hepatic cytosolic glutathione content and the activities of glutathione S-transferase and glutathione reductase, indicating that kimchi might be involved in detoxification of the xenotoxic materials in the liver.

Kimchi extracts also enhanced immune functions of NK (natural killer) cells and macrophages (94). Conceivably, the antimutagenic, anticancer, and antimetastatic activities of kimchi could be increased by manipulating the kinds and levels of the ingredients and the fermentation method (42,103,104). In our studies (see Table 13), the lung metastasis with colon 26-M3.1 cells was significantly reduced by subcutaneous (sc) administration of kimchi extract (0.05 to 1.25 mg/mouse) in mice following the tumor cell inoculation. The functional kimchi that we developed exhibited strong antimetastatic activity.

VII. COMMERCIAL KIMCHI PRODUCTION AND TRADE

A. Commercial Kimchi

The commercial production of kimchi started in the 1960s when it was supplied to the Korean army stationed in Vietnam. Kimchi production has increased considerably for commercial purposes since the 1970s. Kimchi was one of the official foods selected for the 1988 Seoul Olympic games, the 1992 Barcelona Olympic games, and the 1996 Atlanta Olympic games. Koreans consume 1526,000 tons of kimchi per year, of which 115,000 tons (7.5%) was produced by commercial kimchi manufacturers in 1992, increasing to 408,000 tons (27.1%) in 1997, about a fourfold increase in five years. The general consumption of commercially made kimchi has increased by more than 10 times (105). Most kimchi products sold in the domestic market were packaged in plastic bags, pouches, or glass/plastic jars (106). Kimchi products consist of 70 percent baechu cabbage kimchi, 20 percent diced radish kaktugi kimchi, and 10% other (2).

The kimchi industry in Korea has shown rapid growth with the increasing domestic and overseas demand for kimchi. The number of kimchi factories has increased to meet the demand for the domestic supply and for export, but most are small-scale plants. Although

459 kimchi processing factories are now operating in Korea, only a few plants have more than 100 employees.

B. International Trade in Kimchi

Since the 1988 Seoul Olympic Games, the international trade in kimchi has been steadily increasing by 25–30% yearly. Commercial kimchi products are exported to over 33 foreign countries, most of it is exported to Japan because of the frequent cultural exchange between Korea and Japan, as well as the similar dietary tastes and the health benefits of kimchi. The types of kimchi exported consist of 92.3% baechu cabbage kimchi, 6.1% radish kaktugi, 1.0% chonggak kimchi, and 0.6% other (105).

The food inspection criteria for kimchi comply with the Codex standards under the WTO foundation and its SPS agreement (Agreement of Application on Sanitary and Phytosanitary Measures). These measures are needed for the elaboration of the international kimchi standard to protect consumers' health and to ensure fair practices in the food trade. The Codex standardization for kimchi was passed at the 24th Codex Assembly in Geneva, Switzerland in 2001. Thus more kimchi products are likely to be traded worldwide.

VIII. CONCLUSIONS

Kimchi is one of the most popular Korean traditional foods as evidenced by its long history and by the increasing demand for it in other foreign countries. Nutritionally, kimchi is a probiotic food with a distinct taste, and it has a promising future as an excellent functional food. The kimchi recipe calls for high-quality raw materials for better quality kimchi. Elaborated fermentative processes also are needed. For example, a brining process that reduces unnecessary aerobic and pathogenic bacteria, yeasts and molds is needed, but also one that stimulates the growth of LAB. Although starter cultures can be induced for kimchi fermentation, the proper technique for the removal of endogeneous microorganisms, without damaging the texture of the fermented cabbages, has yet to be established. Further research on various fermentation techniques is needed for the optimization of an extended preservation period that increases both functionality and taste. Additional research is needed on modern and appropriate kimchi containers that mimic the old-fashioned earthenware crocks that provided the best kimchi fermentation conditions over a long storage period. The development of new packaging materials and packing techniques that will ensure stable shelf-life are key to the successful internationalization of kimchi. To make kimchi more appealing to foreigners' taste, a variety of kimchis must be developed.

The potential efficacy of kimchi for its anticancer, antiatherosclerotic, weight control, and antiaging properties is interesting and needs more systematic and careful research to establish its potential. The Codex standardization of kimchi that is required for trading allows kimchi to be distributed worldwide, so more intensified research on kimchi processing, fermentation, taste improvement, and nutritional functionality is needed for further development in the quality of kimchi.

ACKNOWLEDGMENTS

The critical review of this manuscript by Dr. B. P. Yu of the University of Texas Health Science Center at San Antonio and the assistance of graduate students of the Department

of Food Science and Nutrition, Pusan National University, in preparing this manuscript are gratefully appreciated.

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36

Jalapeño Pepper Preservation by Fermentation or Pickling

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I. INTRODUCTION

Jalapeño pepper is a widely consumed product in Mexico. This is a scalded, pasteurized product, generally merchandized in cans or glass jars, with brine to which spice has been added. However, Jalapeño pepper shelf-life extension by fermentation or pickling is carried out only at very small industrial level. Information regarding fermented or pickled vegetables is scattered, and there is no clear differentiation between pickled and fermented products (1). This chapter describes the processing of fermented and pickled Jalapeño pepper sold in cans or glass jars.

Pickling and fermentation are preservation methods that extend fruit and vegetable shelf lives via a simple and in expensive technology. The processed material undergoes transformation resulting in a food more acceptable to the consumer.

Pederson (2) pointed out the various methods for fruit and vegetable preservation:

1. Pickling without undergoing fermentation
2. Fermentation in a low-concentration brine
3. Fermentation in a high-concentration brine
4. Preservation by dry salting at low salt concentration

However, there is a controversy regarding whether the terms "pickling" and "fermentation" are equivalent. According to Pederson and Luh (3) pickled products are those to which edible acids have been added, either lactic or acetic (vinegar); on the other hand, fermented products are such that the acid present was produced from sugars by bacterial action. Both pickled and fermented vegetables are mainly preserved by the action of acid, which also improves the product's sensory characteristics and possibly its nutritive value. According to Pederson's classification (2), jalapeño pepper can be either fermented or pickled.

Undesirable microbial growth is inhibited by the acid as well as by salt concentration (4). Besides reduction in the spoilage-causing microbial population, the shelf-life extension of fermented or pickled vegetables also depends on the decrease of inhibition of enzymatic activity of the plant material involved in the ripening process. Control of both spoilage mechanisms in jalapeño peppers, enzymatic and microbial, is achieved by pickling and fermentation.

II. FERMENTED JALAPEÑO PEPPER

This preservation method is based on acid production by fermenting sugars in the plant material through the action of lactic acid bacteria such as *Lactobacillum plantarum*, although the presence of *Leuconostoc mesenteroides* also has a marked effect upon the fermentation and product quality (5,6). In addition of lactic acid bacteria activity, other fermentative bacteria, such as acetic acid–producing microorganisms, also carry out vegetable fermentation, which enhances shelf life and sensory characteristics (6). Undesirable microorganisms are inhibited by various mechanisms. Salt addition allows the growth of naturally present lactic acid bacteria, but the combined salt and acid action allows the

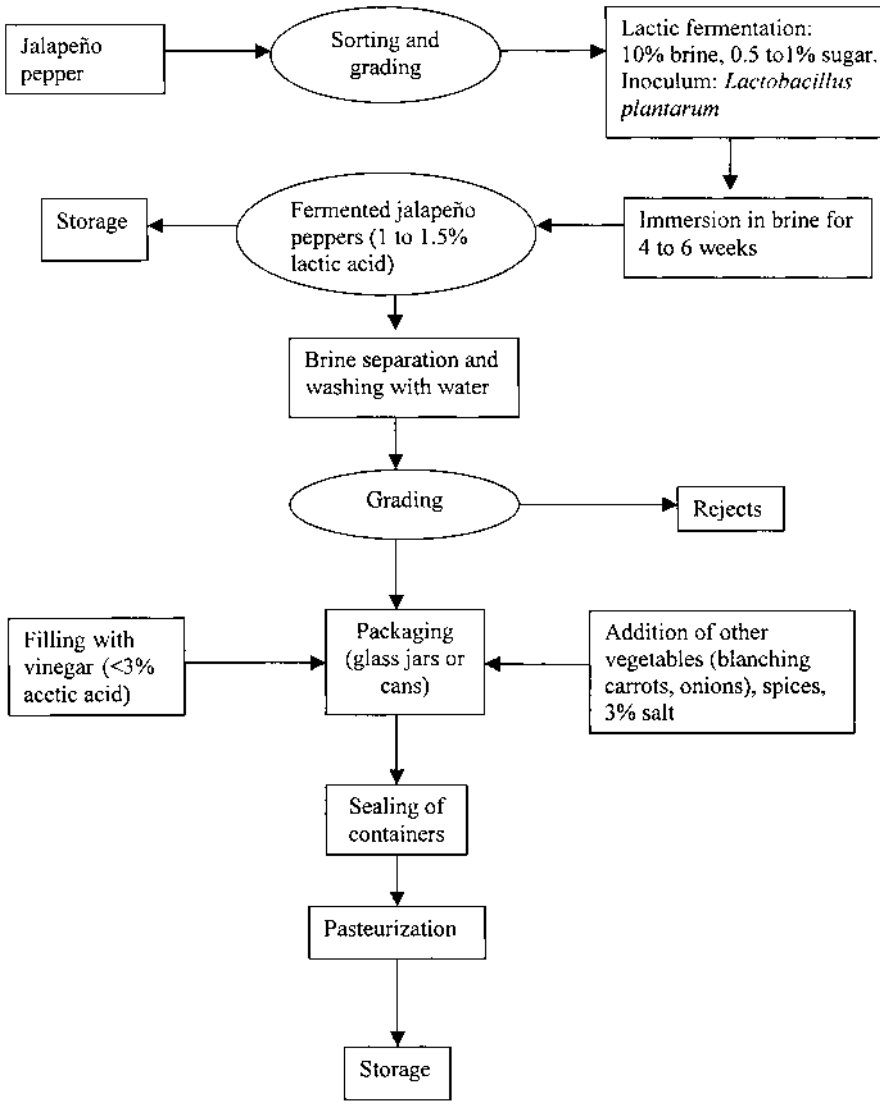

Figure 1 Fermentation of jalapeño pepper.

selection of microflora associated with vegetable preservation. At the same time, fermentation reduced carbohydrate concentration, increasing acid production (7). In some cases, sugars are added to enhance the fermentation process (4). The most important conditions for an adequate vegetable fermentation are: anaerobiosis, salt concentration, temperature, and the used of suitable starters. Lactic acid bacteria can be present as native microflora in the pepper, but to assure a uniform fermentation, selected starters are usually added.

To obtain the best fermented jalapeño pepper quality, the raw material (*Capsicum annuum) cv*. Jalapeño must be recently harvested, still green, and without wounds or peduncle. Figure 1 shows the general flow diagram of fermented jalapeño pepper processing (8).

A. Preliminary Operations

Raw jalapeño peppers are selected according to their size and quality. They are washed, and small incisions are made in order to facilitate brine diffusion to the central part and to eliminate gas formed during fermentation. Washing diminishes the hot pepper fermentative ability; so it is necessary to add a starter culture (*Lactobacillum plantarum*). It is the same for pepper blanching.

III. FERMENTATION

Fermentation is carried out by facultative anaerobic homofermentative strains such as *Lactobacillum plantarum* and *Pediococcus cereviseae*. *L plantarum* produces acetic and lactic acids as well as ethanol and gas (CO_2 and H_2). The peppers are then immersed in 10% brine for 4 to 6 weeks, sometimes with 0.5 to 1% sucrose as well as hot pepper cell fluid containing carbohydrates, nitrogen compounds, and minerals among other things. The cell fluid from the peppers, however, tends to dilute the brine. For this reason, it is necessary to add 1% salt daily during the first week, and three times a week during the rest of the immersion time, in order to keep the desired brine concentration (18–20%). The peppers must be completely covered by the brine at all times.

Fermentation takes place in 4 to 6 weeks. It is carried out in closed tanks, with a vent to allow gas formed during the process to dissipate. At the end of the fermentation period, the peppers, originally bright green, turn into olive green. The plant tissue also changes, taking on a translucent aspect. Acid concentration increases from 0.8 to 1.5% (expressed as lactic acid), promoting a decrease in pH. The peppers are then washed to eliminate salt excess, classified according to their size, placed in glass jars or plastic bags, mixed with other vegetables, usually carrots and onions, and covered with vinegar.

Fermented jalapeño peppers are highly perishable if the vinegar has less than 3% acetic acid. In this case, pasteurization is necessary. It is carried out over 30 min at 71°C (for glass jars containing 280 g of product). Finally, the product is labeled, packaged, and stored in a similar way as for pickled (nonfermented) jalapeño peppers.

IV. PICKLED (NONFERMENTED) JALAPEÑO PEPPERS

Jalapeño peppers that are most widely sold in producing countries, such as Mexico are pickled nonfermented products. They are sold in different can sizes and consist of whole

cut peppers, mixed with scalded onions, carrots and mushrooms, with vinegar to which spice has been added. (Fig. 2).

The main difference between this product and fermented peppers is that the raw material is fresh peppers or peppers preserved with salt (brine). According to its acidity, the product is then heat-treated (9). If it is an acidic food, spoilage can takes place, so a further preservation method is necessary. The flow diagram for this process is shown in Fig. 3.

At the domestic level or in small industries, pickled jalapeño peppers are processed by mixing scalded carrots, onions, and other vegetables with jalapeño peppers cut lengthwise, and adding vinegar previously flavored with pepper, cinnamon, marjoram, thyme, and clove, and other condiments (onion, garlic, and laurel fried in vegetable oil) (10).

A. Preliminary Operations

Contaminants or inedible components can be present when jalapeño peppers, carrots, and onion are gathered for processing. Therefore it is necessary that the vegetables undergo one or more of the following: washing, selection, classification, size reduction, and scalding. Canning also ensures an adequate edible quality of the product.

After jalapeño peppers, carrots, and onions are then transported into the processing plant, the vegetables are selected for processing or storage, according to their quality and stage of ripeness. Washing by immersion, spraying, or combined methods is done in order to eliminate various contaminants (11,12).

1. Washing

During immersion washing, the dirt that adheres to the vegetable surface are softened and eliminated together with stones, sand, and other abrasive material, which can damage the equipment during further operations. The immersion tanks are made of metal, mortar, or building materials suitable for easy cleaning and disinfections. In order to improve the washing efficiency, stirring is provided. Detergents or chlorine is also added to decrease the microbial load. During spray drying, vegetables are exposed to pressurized water, which is applied when the water supply is restricted.

2. Sorting and Grading

These operations are performed to discard products unsuitable for processing: damaged, unripe, overripe, or deformed vegetables. Deterioration occurs during harvesting, transportation to the processing plant, or cleaning.

Vegetables are selected according to size and quality and are directed to processing or direct consumption. In general, classification consists in simultaneous evaluation of various physical properties. In the case of hot peppers, onions, and carrots: classification is carried out manually. By this procedure it is possible to have a simultaneous evaluation of several attributes that would be difficult to evaluate in an automatic fashion. The advantage of classification is having uniform material to be directed to a specific operation, such as peeling, size reduction, or blanching. Grading also homogenized the product, improving heat-processing efficiency. Trained personnel generally carry out quality classification.

3. Peeling

Peeling is a necessary operation during carrot and onion processing; to improve the product appearance, the elimination of inedible parts must be carried out, although it is

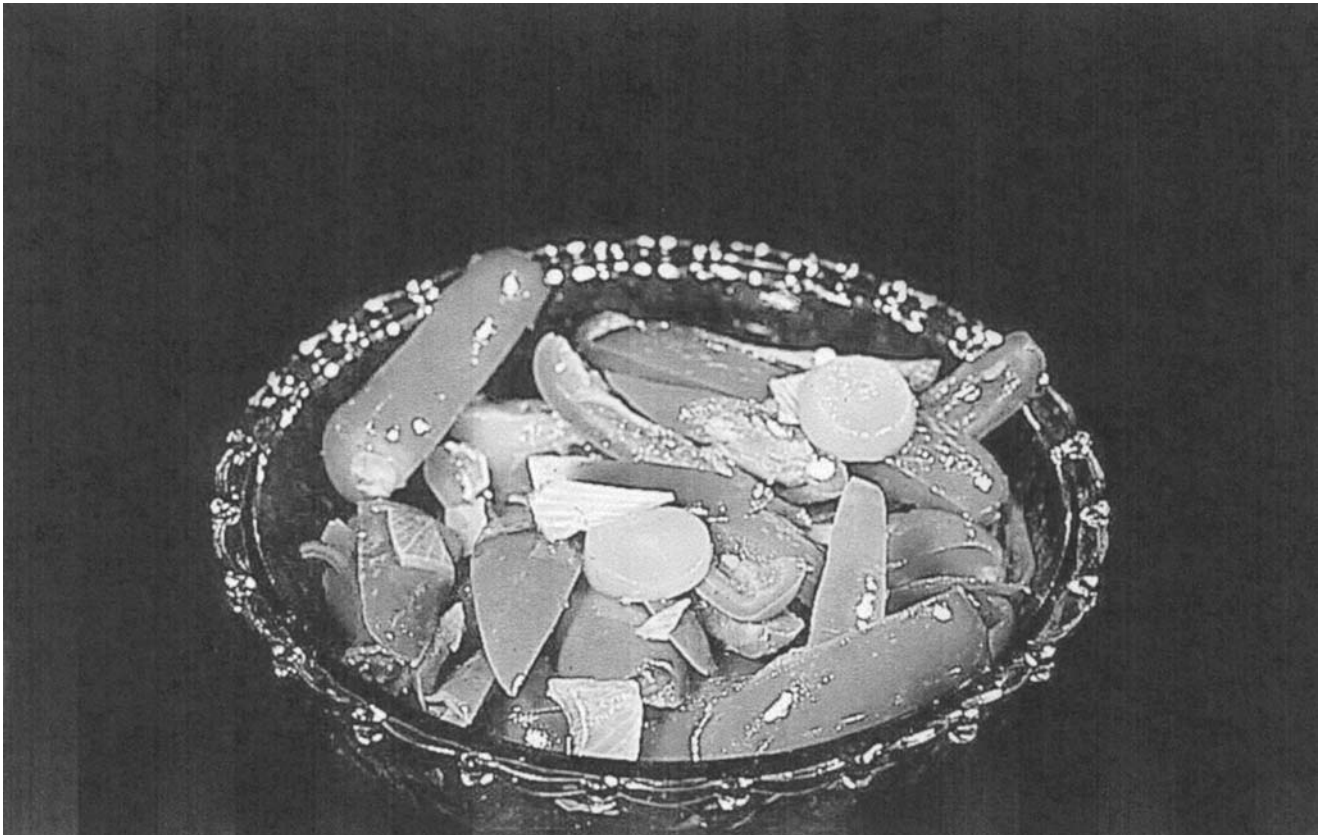

Figure 2 Pickled and cut jalapeño peppers.

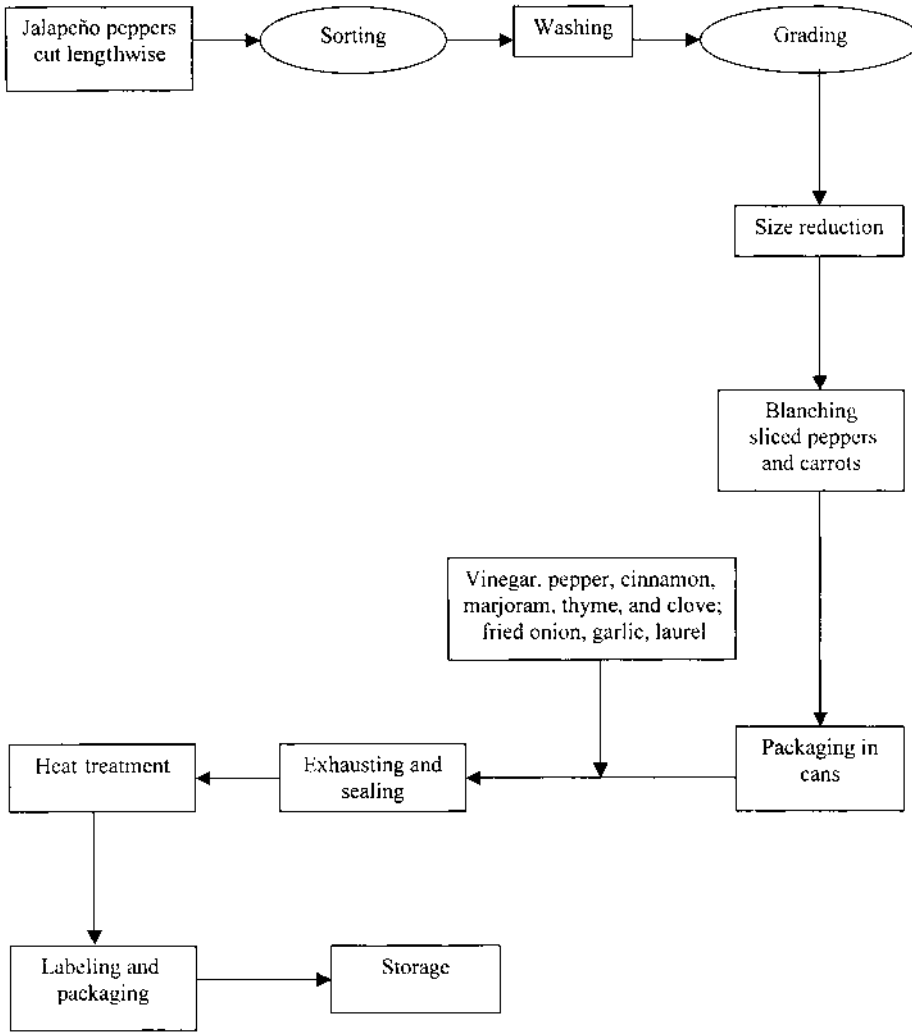

Figure 3 Jalapeño pepper: nonfermented pickling.

important not to eliminate large portions of the vegetable. There are different peeling methods:

Abrasion: Carrot peeling is done by abrasion. In this way, skin is removed by friction; the product is in contact with carborundum rollers or placed into containers with the inside coated with an abrasive material such as silicon or carbon. The abrasive surface detaches the carrot skin, which is later removed by a water stream.

Flame peeling: Applied mainly to onions, it consists of placing the vegetables on a transportation band moving through an oven at >1000°C. As the vegetables pass, the outmost layer and fine roots are burnt and eliminated by high pressure water spraying.

4. Size Reduction

In this operation, the average size of a solid food material is reduced by the application of external forces such as impact, compression, or abrasion (11). In the case of jalapeño peppers, they are cut lengthwise into four parts and the peduncle and seeds are eliminated. Cutters consist of a series of rotating blades, centrifugal forces holds the product against the blades.

5. Spoilage Enzymes

Enzymes, endocellular, exocellular, or microbial, assume an active role in food deterioration. Microbial enzymes are also able to act on the food substrate even when the microbial cell is inactivated or dead (13). Insufficient scalding can result in an increase in food spoilage as heat applied can disrupt the tissues, liberating the substrate but not inactivating the enzymes. Scalding efficiency in vegetables is measured by inactivation of two enzymes: catalase and peroxidase. In processed jalapeño pepper, the time–temperature conditions for scalding are (9) jalapeño pepper: 8 to 10 min, water at 95°C; carrots: 6 to 8 min, water at 95°C.

6. Blanching

This heat process is applied prior to processing in order to inhibit enzymatic activity or decrease microbial populations. Blanching can be combined with other operations such as peeling or cleaning (11–13). An efficient enzyme inactivation is achieved by heating until calculated temperature–time conditions are reached and then fast-cooling to room temperature. The two blanching methods commonly used are saturated steam and immersion in hot water. At industrial levels, steam blanching is the most widely practical method (11). It consists in applying steam to the vegetables on a conveyor belt going though a steam tunnel. Varying the speed of the conveyor belt controls the time of residence in the tunnel. In some cases a water spray is applied at the start and end of the conveyor in order to condense excess steam. During hot water blanching, the vegetables are held for a given time at 70–100°C, with a further draining–cooling period afterwards.

B. Packaging

The aim of this operation is to keep the product, from processing to the consumer, in the same hygienic and quality conditions. Cans are made from three-piece tin sheets, coated on the inside with epoxyphenolic enamel (Fig. 4). The lids also made of tinfoil and coated with the same epoxyphenolic enamel that is used in the can. The lids also have two or three circular expansion rings, providing resistance against deformation due to an increase in the internal pressure (14).

C. Pickle

According to Mexican regulations (15), pickle is a mixture of vinegar, vegetable oil, onion, carrots, laurel, garlic, salt, sugar, and spices. The last ingredient is optional. Vinegar includes 2% acetic acid and 5% sodium chloride.

D. Blanching Vegetables

Cut peppers, carrots, and onions must be approximately 60% of total product weight, being peppers having a higher proportion.

Figure 4 Three-sheet tin cans covered inside with porcelain enamel.

E. Filling

Vegetable mix is first added to the can, previously washed with hot water; the brine (pickle) is then added at 82 to 85.6°C. Filling must be carefully controlled in order to assure that the correct amount of vegetable mix and pickle is added, and to fulfill specifications. Headspace must be 10% of total can volume. Filling is done when transported by the conveyors, which carry the cans to the vegetable mix filler and then to the liquid one.

F. Exhausting

Exhausting: When air is evacuated from the headspace before sealing, internal pressure is decreased during sterilization. At the same time, oxygen evacuation prevents tin corrosion and oxidation. During this operation, air is replaced by vapor, producing partial vacuum in the headspace after condensing. Exhaustion is carried out in tunnels (or exhausters), as shown in Fig. 5. Another way to promote exhaustion is by using steaming machines, which inject steam into the headspace before closing the cans (14).

G. Sealing

Sealing is carried out in a seaming machine. According to the design and speed of the operation, the basic stages of the operations are as follows: (a) the edges are folded; (b) the folded tin is pressed to form a hermetic seal, impermeable to air (16).

H. Heat Treatment

Cans or glass jars are subjected to heat treatment to sterilize or pasteurize their contents. It can be done in batches or by continuous retorting. Cans are heated at a time–temperature condition in vapor or hot water. Pasteurization of pickled jalapeño peppers destroys microorganisms resistant to high acetic acid concentrations, able to promote product alteration. Heat treatment also inhibits vegetable or microbial enzymes (17). Heat treatment of 93.3°C and 10 min are recommended for acid pickles (pH 4.3 to 4.5). However, a time–temperature process depends on the type of container, the volume, and the heat processing equipment.

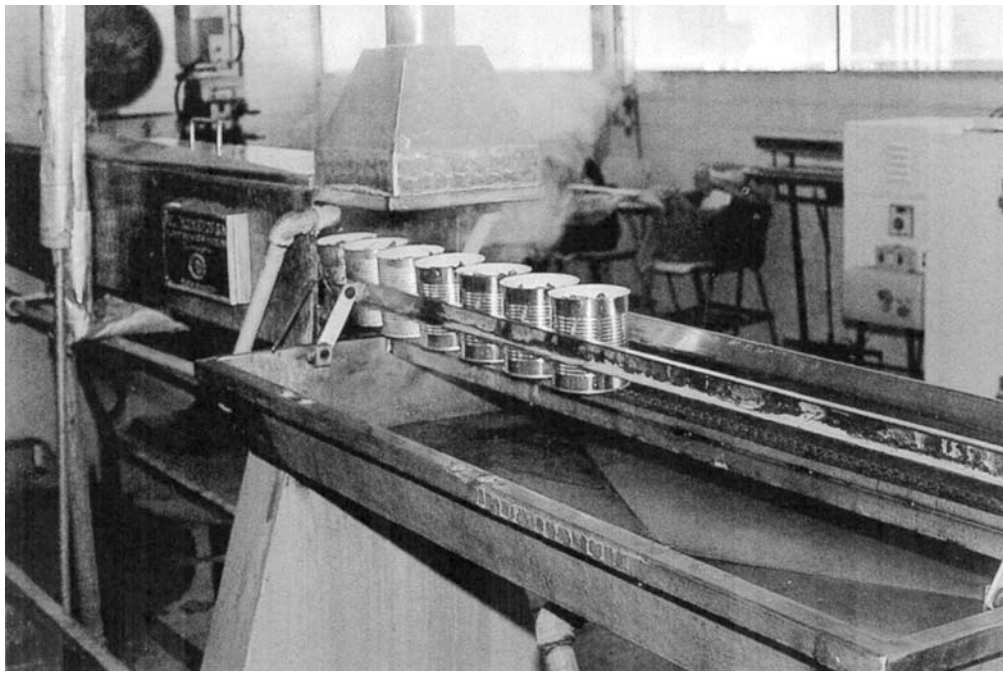

Figure 5 Vapor tunnel or exhauster.

1. Batch Processing

During this operation retorts are saturated with vapor and containers are placed in baskets. Retorts can be horizontal or vertical, and the cans can be still or rotating during the process. Can rotation promotes heat transfer so that processing time is reduced and higher temperatures can be achieved.

2. Continuous Retorting

This type of equipment is fitted with hydrostatic closings before and after the pressurized sections. Processing can be also carried out by can rotation, where the cans move in and out of the pressurized section through hydrostatic water column seals, which equilibrate the internal pressure.

A variation of this equipment is the flame retort, working at atmospheric pressure throughout the operation. Flame retort equipment is fitted with direct heating, applied to the rotating retort. An advantage of this type of retorting is a high product quality due to mild heating conditions.

In all heat treatments, the final part is cooling the can with water to reach a final temperature not less than 38°C. Because the cans are not completely cooled down, water is eliminated from the outside, avoiding corrosion.

I. Marking, Labeling, and Packaging

Once the containers undergo heat treatment, each can or jar is marked with a code, a production date, a batch number, and a plant code. The label includes the product name,

the commercial name, the drained and net weight, the ingredients, and other specifications required by the country's regulations (15). Packing is automatically carried out in cardboard boxes or high-density polyethylene bags, or other suitable packaging material with enough resistance to protect the product and containers.

J. Storage

Heat-treated jalapeño peppers keep their quality characteristics at 18 to 21°C. At a higher temperatures, acid products in cans without inner coating consume oxygen in the headspace faster than in coated cans. The result is a considerable loss in ascorbic acid content and fast product oxidation (17,18). On the other hands, canned jalapeño peppers have a longer shelf life if stored at 0 to 5°C (17).

V. REGULATIONS

A. Mexican Specifications

Mexico has quality bylaw (Norma Official) regulations for pickled jalapeño or Serrano peppers (NOM-F-121-1982, 15). This regulation includes six consumer presentations and two quality levels.

The presentations are whole peppers, peppers without seeds, peppers in halves, peppers cut lengthwise, peppers cut in rings, and chopped peppers. There are two quality classifications for whole peppers only (minimum and maximum); for the rest of the presentations there is one quality classification. Table 1 shows physical and chemical specifications.

These specifications also include microbial characteristics, chemical contaminants, optional ingredients, sampling and specificity of quality degrees, labeling, containers, and packaging. Among optional ingredients are garlic, pepper, cinnamon, cloves, ginger, laurel, marjoram, thyme, and nutmeg. In defining the Mexican official specifications the main jalapeño pepper processing industries took part, such as Productos Del Monte, La Costeña, Herdez, Conservas San Miguel, Conservas Guajardo and Elías Pando.

B. International Specifications

The processed fruit and vegetable Committee of the Codex Alimentarius Commission FAO/OMS has elaborated a General Specification project for pickled products. At present, this project is at the sixth stage, that is, revision by all member countries. However, the project does not include pickled cucumbers or kimchi (19).

Table 1 Specification of Jalapeño Peppers (NOM-F-121-1982, 15)

Specification	Minimum	Maximum
Acidity (as acetic acid) (%)	0.75	2.0
Chlorides (as sodium chloride) (%)	2.0	7.0
pH	—	4.3
Filling (%)	90	—
Headspace (%)		10
Vacuum (mm Hg)	76.2	—

ACKNOWLEDGMENTS

The author thanks Dr. Isabel Guerrero Legarreta for the manuscript revision.

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Sauerkraut

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I. INTRODUCTION

Sauerkraut or kraut is prepared from sound, well-matured heads of the cabbage plant (*Brassica oleracea* var. *capitata* L.) which have been properly trimmed and cut; to which salt (2 to 3%) is added and which is cured by natural fermentation. The finished product contains not less than $1\frac{1}{2}$ % of acid, expressed as lactic acid. The product may be canned by processing sufficiently by heat to assure preservation in hermetically sealed containers, or if may be packaged in sealed containers and preserved with or without the addition of benzoate of soda or any other ingredient approved by the FDA (1).

II. SAUERKRAUT PRODUCTION

A. Cabbage Varieties

A number of cabbage varieties have been used for sauerkraut production. The five commonly used commercial varieties are Dutchmaster Ferry Morse 6201, King Cole, Roundup, Glory 61, and Large Glory. New hybrids of cabbage suitable for sauerkraut production have been investigated (2).

B. Chemical Composition of Cabbage

Starner et al. (2), in analyzing eleven fermentable cabbage varieties, found 35.0 to 49.0% fermentable carbohydrate, 2.3 to 3.5% protein nitrogen, 7.0 to 9.0% ash, 0.28 to 0.45% inorganic phosphorus, 0.27 to 0.49% inorganic sulfor, and 5.3 to 7.1% solids.

C. Sauerkraut Process

The sauerkraut process is described by Hang et al. (3) as follows: the cabbage is delivered to the factory by truck. It is then transported via conveyor to the coring machine. Following this, the cored head is conveyed to the trunning table where outer leaves and bad spots are removed. This latter operation represents a source of solid wastes.

Next the cabbage is shredded and transported to the fermentation vat. Salt, 2.25 to 2.5 kg per 100 kg of cabbage, is applied evenly as the shreds are distributed in the vat. Juice is released from the cabbage almost immediately after addition of the salt. To assure a maximum fill of cabbage into a vat, much of this "early brine" may be withdrawn from the vat and discarded during or shortly after the filling. This early brine can be a significant source of liquid waste in the sauerkraut process.

After the vat is filled, it is covered with a plastic sheet that is weighted with water. The fermentation is considered complete when the titratable acidity, expressed as lactic acid, has reached 1.5%, and the shreds are fully cured. This requires four or more weeks.

The final step is the filling of the fermented cabbage and brine into retail packages (cans, jars, or flexible pouches). Only a portion of this "late brine" is added to the packages; the remainder, which must be discarded, represents a second important source of liquid waste.

D. Microorganisms Involved in Sauerkraut Fermentation

Sauerkraut fermentation is a complex microbiological process involving a sequence of growth of various types of microorganisms. Raw cabbage contains sufficient numbers of desirable lactic acid bacteria for spontaneous fermentation. In the early stage of fermentation, most of the lactic acid bacteria are the heterofermentative (gas-forming) species such as *Leuconostoc mesenteroides*. The carbon dioxide creates an anaerobic environment that promotes the growth of desirable lactic acid bacteria but excludes the presence of oxidative fungi. After 8 days of fermentation, most of the lactic acid bacteria are the homofermentative (nongas-forming) species such as *Lactobacillus plantarum* (4). Other homofermentative lactic acid bacteria, *Lactobacillus brevis* and *Pediococcus cerevisiae*, also play an important role in the conversion of shredded cabbage to sauerkraut of high quality (5).

E. Chemical Changes During Sauerkraut Fermentation

Cabbage contains 4.69% total sugars (0.25% sucrose, 2.38% glucose, and 2.05% fructose). During fermentation, sugars were rapidly converted to lactic acid by the lactic acid bacteria present on raw cabbage. Other important products formed during the fermentation are carbon dioxide, mannitol, acetic acid, and ethanol (4).

F. Factors Affecting Sauerkraut Fermentation

It has been shown that growth and fermentation patterns are affected by (a) the variety of cabbage, (b) the temperature, and (c) the salt concentrations.

1. Variety of Cabbage

Stamer et al. (2) evaluated the ability of 13 varieties of cabbage, including 5 commonly used commercial varieties and 8 newly developed hybrids, to undergo lactic acid fermentations. Eleven of the 13 varieties could undergo normal fermentation, but two varieties (G27 × G51 and G60 XW-1) failed to support adequate fermentation and consistently resulted in sauerkraut of poor quality. The inability of these varieties to undergo normal fermentation may be due to the presence of growth inhibitory substances or the lack of nutritional factors essential for the growth of lactic acid bacteria.

2. Temperature

The temperature of shredded cabbage has a profound influence on the rate of fermentation. The heterofermentative species, *Leuconostoc mesenteroides*, that initiates the fermentation can grow at relatively low temperatures (5). At 7.5°C, for example, this organism produces 0.4% and 0.8 or 0.9% lactic acid in about 10 days and less than a month, respectively. At 18°C, *Leuconostoc mesenteroides* and other homofermentative species, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Pediococcus cerevisiae*, grow in a natural sequence and can produce more than 1% lactic acid in a few weeks and about 2% in about 2–3 months. The rate of sauerkraut fermentation is rapid at 32°C and 1.8 to 2% lactic acid can be produced in 8–10 days. In general, sauerkraut fermented at lower temperatures has a better color, flavor, and character than sauerkraut fermented at higher temperatures.

3. Salt Concentrations

It is important to add a proper amount of salt to the shredded cabbage prior to fermentation. Salt is added to withdraw nutrients from cabbage for proper fermentation and to inhibit the growth of undesirable microorganisms. A salt concentration of 2.25% favors the growth of desirable lactic acid bacteria in their natural sequence and results in a finished product with the proper balance of salt to acid (5). A salt concentration of 3.5% or more is detrimental to the growth of the heterofermentative species. *Leuconostoc messenteroides*, that initiates sauerkraut fermentation. Excessive salt causes a significant reduction in the rate of acid production and yields a sauerkraut with an undesirable ratio of salt to acid. A salt concentration of less than 2% can cause some softening of the finished product due to the activity of pectinolytic enzymes.

G. Types of Spoilage

1. Pink Kraut

Pink kraut poses considerable economic losses to food processors. Pink kraut is caused by a condition associated with excess salt in the fermentation tank. Yeasts have been reported to impart color to kraut (6). Gorin and Jans (7) have reported that the color in pink kraut is probably attributable to the formation of a leucoanthocyanidin. Stamer, et al. (8) have shown that *Lactobacillus brevis* plays an important role in the production of a water-soluble red pigment that is presumably responsible for imparting a highly objectionable discoloration to sauerkraut.

2. Soft Kraut

Soft kraut is caused by a condition associated with insufficient salt. Sufficient salt must be added to draw enough juice from the shredded cabbage to promote the growth of desirable lactic acid bacteria in their proper sequence. Lactic acid and salt are required to prevent the softening of sauerkraut due to the activity of pectinolytic enzymes.

3. Rotted Kraut

Rotted kraut may be caused by the presence of undesirable microorganisms. To exclude air and to prevent the growth of undesirable types of bacteria, yeasts and molds, it is important to cover the shredded cabbage with a plastic sheet at the time of packing and place water in the plastic cover to cause it to be immersed in the brine.

4. Off-Flavor Kraut

Off-flavor kraut may be caused by too rapid a fermentation at high temperatures. Aerobic yeasts and molds can also grow and produce undesirable flavors and odors. The finished product is of inferior quality. The product is like esters kraut and usually has low concentrations of acetic acid, ethanol, and esters.

III. CHEMICAL ANALYSES (9)

A. pH

The pH of sauerkraut brine is determined with a pH meter. Sauerkraut has a pH range of about 3.3 to 3.8.

B. Titratable Acidity

The titratable acidity of sauerkraut brine expressed as lactic acid is determined by titrating the sample with 0.1 N NaOH using phenolphthalein as an indicator or titrating it to pH 8.2 with a pH meter. For example, the titratable acidity of a 10 mL sample can be calculated by mL of 0.1 N NaOH used $\times$ 0.090 = % titratable acidity expressed as lactic acid.

C. Salt (Sodium Chloride)

The salt content of sauerkraut brine is determined by titrating the sample with 0.171 N silver nitrate using 0.5% dichlorofluorescein as an indicator. One mL of 0.171 N silver nitrate is equal to 1 g of salt per 100 mL of sample.

IV. PROCESSING OF SAUERKRAUT

The finished product is heated in hot brine to above 74°C (165°F) and placed in cans, glass jars, or plastic bags. The filled containers are then conveyed to an exhaust box and to a container sealer. The sealed containers are water-cooled to about 38°C (100°F) and then stored in a cool place (10).

V. QUALITY FACTORS AND GRADES FOR SAUERKRAUT (1)

Factors that affect the quality of sauerkraut are color, cut, defects, character, and flavor. The relative importance of each factor rating is expressed numerically on the scale of 100. The maximum number of points that may be given each such factor is color, 30; cut, 10; detects, 20; character, 10; and flavor, 30.

The USDA Standards for Grades recognize four U.S. grades of canned kraut. The grades are "U.S. Grade A" or "U.S. Fancy," "U.S. Grade B" or "U.S. Extra Standard," "U.S. Grade C" or "U.S. Standard," and "Substandard."

U.S. Grade A or U.S. Fancy is the quality of kraut that possesses a good color; that is well cut; that is free from defects; that possesses a good character; that possesses a good flavor; and that, for those factors that are scored in accordance with the scoring system (Table 1), the total score is not less than 90 points.

Table 1 Score Points for USDA Standards for Grades for Sauerkraut

Factor		Score points for grade			
		A	B	C	Substandard
Color	30	27_30	24_26	21_23	0_20
Cut	10	9_10	8	7	0_6
Defects	20	18_20	16_17	14_15	0_13
Character	10	9_16	8	7	0_6
Flavor	30	27_30	24_25	21_23	0_20

Source: Ref. 1.

U.S. Grade B or U.S. Extra Standard is the quality of kraut that possesses a reasonably good color; that is reasonably well cut; that is reasonably free from defects; that possesses a reasonably good character; that possesses a reasonably good flavor; and that, for those factors that are scored in accordance with the scoring system, the total score is not less than 80 points.

U.S. Grade C or U.S. Standard is the quality of kraut that possesses a fairly good color; that is fairly well cut; that is fairy free from defects; that possesses a fairly good character; that possesses a fairly good flavor; and that, for those factors that are scored in accordance with the scoring system, the total score is not less than 70 points.

Substandard is the quality of canned kraut that fails to meet the requirements of U.S. Grade C or U.S. Standard.

VI. WASTE MANAGEMENT

A. Material Balance of Sauerkraut Production

Data on material balance (Table 2) show the quantity of sauerkraut produced from 100 tons of cabbage. It can be seen that about 29% of the salted shredded cabbage is discarded as brine (liquid waste). The data also show that there is a problem of solid as well as liquid waste

Table 2 Material Balance of Sauerkraut Production

Materials	Tons
Raw cabbage	100.00
Solid waste (trim loss)	35.30
Shredded cabbage in vat	64.70
Salt added	1.70
Liquid wastes	
Early brine	11.00
Late brine	8.50
Total	19.50
Yield of sauerkraut	46.90

Source: Ref. 3.

Table 3 Characteristics of Sauerkraut Wastes

Source	pH	COD (mg/L)	BOD (mg/L)	Lactic acid (mg/L)	NaCl (mg/L)	Total N (mg/L)	Total P (mg/L)
Vat soak water	7.5	64	60	—	—	—	—
Vat wash water	10.4	303	236	—	—	—	—
Early brine	5.2	17,730	11,100	926	36,800	555	106
Late brine	3.5	28,960	24,300	18,600	28,600	1,090	189

Source: Ref. 3.

in the manufacture of sauerkraut. The trim loss of 35.3 tons is an average of 16 different loads of cabbage; individual values ranged from 28.7 to 41 tons. The solid wastes are generally returned to the growing field. The average yield of sauerkraut is 46.9 kg per 100 kg of cabbage fermented.

Data in Table 3 show the characteristics of liquid wastes generated in sauerkraut production. In addition to early and late brines, other sources of waste effluents are the vat soak water, vat wash water. Because of their low BOD values, both vat soak water and vat wash water should be readily biologically treatable. The surplus sauerkraut brines present the greatest problem with respect to treatment because of their strength. These high BOD brines may require their segregation for separate treatment.

B. In-Plant Treatment of Liquid Waste

As shown in Table 2, about 20 tons of brine are generated by fermentation of 100 tons of shredded cabbage. This is characteristic of the sauerkraut process. The discarded brine poses serious environmental problems because of its high BOD, acid, and salt contents. A simple yeast process has been developed for in-plant treatment of liquid wastes (11,12). The sauerkraut wastewater is treated in a bioreactor with a food yeast under aerobic conditions. Upon completion of the treatment, the mixed liquor is allowed to settle, and the supernatant fraction can be discharged to a municipal waste treatment plant. The settled yeast cells can serve as an inoculum for treatment of another batch of sauerkraut wastewater. The yeast is capable of rapidly converting the organic matter to yeast cells and thus can reduce the BOD by as much as 93%. The reductions of nitrogen and phosphorus are more than 75 and 90%, respectively. As a result of metabolism of the lactic acid by the yeast, the sauerkraut waste effluent is neutralized. This has economic significance, since acid waste effluents generally require neutralization before they can be treated in a secondary waste treatment system such as an activated studge process or a trickling filter.

VII. BY-PRODUCT RECOVERY

A. Food Yeast

Sauerkraut brine is a favorable substrate for the cultivation of food yeasts. *Saccharomyces cerevisae*, *Candida utilis*, and *Kluyveromyces fragilis* (13). The brine contains sufficient nutrients for support of yeast growth. The yield of yeast cells is greater than 65 g per 100 g of the BOD removed. The freeze-dried cells of the yeast, *Candida utilis*, for example, contain 40.80% protein, 0.65% fat, 43.41% carbohydrates, 6.18% ash, 6.20% fiber, and 2.76% moisture.

B. Enzymes

Food yeasts grown in sauerkraut brine have been reported to exhibit considerably high activities of beta-fructofuranosidase, beta-galactosidase, acetoin reductase, and diacetyl reductase (14). These enzymes may have value in a variety of commercial applications.

C. Carotenoids

Caretenoids can be produced by *Rhodotorula rubra* NRRL Y-15596 from sauerkraut brine under controlled growth conditions. The maximal yield of caretenoids expressed as beta-carotene is 131 mg per g of yeast dry weight or 1041 mg per liter of sauerkraut brine with an initial BOD value of 11,000 mg/liter (15).

VIII. CURRENT DEVELOPMENTS

Lactic acid and acetic acid are the major products of sauerkraut fermentation, and their concentrations in the finished product are highly variable. Research is needed for improvement in the uniformity of sauerkraut quality. Possible methods for controlling the level of acidity in the finished product include (a) pasteurization of the product when it reaches the desired acidity, (b) development of cabbage varieties with a low concentration of fermentable sugars, (c) dilution of the product with water to the desired level of acidity, (d) neutralization of the finished product, and (e) controlled fermentation and storage of sauerkraut (Fleming and McFeeters, Ref. 16).

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Pickle Manufacturing in the United States: Quality Assurance and Establishment Inspection

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I. QUALITY ASSURANCE

A. Introduction

This chapter is not designed to explain how pickles are manufactured in the United States. Rather, it is designed to show you the critical factors you should look for in assuring the quality of your pickles. The information has been modified from a document issued by the U.S. Department of Agriculture, U.S. Standards for Grades of Pickles. Consult the original document for complete details.

This section contains 11 tables numbered 1 to 11, and they do not appear in the same order as the text references them.

B. Product Description

Pickles are a product prepared entirely or predominantly from cucumbers (*Cucumis sativus* L). Clean, sound ingredients are used that may or may not have been previously subjected to fermentation and curing in a salt brine. The product is prepared and preserved through natural or controlled fermentation or by direct addition of vinegar to an equilibrated pH of 4.6 or below. The equilibrated pH value must be maintained for the storage life of the product. The product may be further preserved by pasteurization with heat or refrigeration and may contain other vegetables, nutritive sweeteners, seasonings, flavorings, spices, and other permissible ingredients as defined by the U.S. Food and Drug Administration (FDA). The product is packed in commercialy suitable containers to assure preservation.

C. Styles of Pickles

1. Whole style means the pickles are whole and are relatively uniform in diameter as indicated in a latter discussion (Table 2).
2. Whole, mixed sizes style means the pickles are whole pickles of mixed sizes.

3. Sliced lengthwise style means the pickles are cut longitudinally into halves, quarters, or other triangular shapes (spears, strips, or fingers), or otherwise into units with parallel surfaces with or without ends removed.
4. Sliced crosswise, crosscut, or waffle cut style, means that the pickles are cut into slices transversely to the longitudinal axis. The cut surfaces may have flat-parallel or corrugated-parallel surfaces.
5. Cut style means the pickles are cut into chunks or pieces that are of various sizes and shapes.
6. Relish style means finely cut or finely chopped pickles containing no less than 60 percent of cucumber ingredient and may contain other vegetable ingredients (cauliflower, onions, pepper, tomatoes, cabbage, olives, mustard, or any other suitable vegetable).

D. Types of Pack

1. Cured Type

The pickles are cured by natural or controlled fermentation in a salt brine solution and may contain the dill herb or extracts thereof. The pickle ingredient may be partially desalted. The pickles may be further processed or preserved by the addition of vinegar and may contain other ingredients (spices, flavorings, firming and preserving agents) that constitute the characteristics of the particular type of pickle. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. The characteristics of the various types of cured pickles are as follows:

1. Dill pickles (natural or genuine) are cucumbers that are cured in a brine solution with dill herb and other flavoring agents.
2. Dill pickles (processed) are brine-cured pickles that have undergone a freshening process and are packed in a vinegar solution with dill flavoring and other flavoring agents.
3. Sour pickles are cured pickles that are packed in a vinegar solution with or without spices.
4. Sweet pickles and mild sweet pickles are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s).
5. Sour mixed pickles are cured pickles that are packed in a vinegar solution. The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 1 or any other suitable vegetable.
6. Sweet mixed pickles and mild sweet mixed pickles are cured pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The pickles may be of any style or combination of styles other than relish and may contain other vegetable ingredients as outlined in Table 1 or any other suitable vegetable.
7. Sour mustard pickles or sour chow chow pickles are cured pickles of the same styles and ingredients as sour mixed pickles except that the pickles are packed in a prepared mustard sauce of proper consistency with or without spices and flavorings.
8. Sweet mustard pickles or sweet chow chow pickles are cured pickles of the same styles and ingredients as sweet mixed pickles except that the pickles are packed in a sweetened prepared mustard sauce of proper consistency with or without spices and flavorings.

Table 1 Proportions of Pickle Ingredients in Certain Types and Styles

Pickle ingredients and styles	Cured, fresh-pack, and refrigerated types (percentage by weight of drained weight of product)	
	Sour mixed; sweet mixed; mild sweet mixed; sour mustard or sour chow chow; sweet mustard or sweet chow chow	Sour pickle relish; sweet pickle relish; dill relish; hamburger relish; mustard relish
Cucumbers, any style other than relish	60 to 80%	—
Cucumbers, chopped or finely cut	—	60 to 100%
Cauliflower pieces	10 to 30%	—
Cauliflower, chopped of finely cut	—	30% maximum (optional)
Onions, whole (maximum diameter of 1–$\frac{1}{4}$ inches), sliced or cut	5 to 12%	—
Onions chopped or finely cut	—	12% maximum (optional)
Green tomatoes, whole or pieces	10% maximum (optional)	—
Green tomatoes, chopped or finely cut	—	10% maximum (optional)
Red, green, or yellow peppers or pimientos, cut, finely cut or pieces	Optional	Optional
Celery	Optional	Optional
Cabbage	Optional	Optional
Olives	Optional	Optional
Tomato paste	Optional	Required in hamburger relish
Mustard or prepared mustard	Required in chow chow and mustard pickles	Required in mustard relish, optional in hamburger relish

9. Sour pickle relish consists of finely cut or chopped cured pickles that are packed in a vinegar solution. Sour pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 1 and may contain a stabilizer such as a starch or gum.
10. Sweet pickle relish and mild sweet pickle relish are finely cut or chopped cured pickles that are packed in a vinegar solution with a suitable nutritive sweetening ingredient(s). Sweet pickle relish and mild sweet pickle relish may contain other chopped or finely cut vegetable ingredients as listed in Table 1 and may contain a stabilizer such as a starch or gum.
11. Hamburger relish consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 1 with tomato product added.
12. Mustard relish consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 1.
13. Dill relish consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 1.

2. Fresh-Pack Type

The pickles are prepared from uncured, unfermented cucumbers and are packed in a vinegar solution with other ingredients to produce the characteristics of the particular type of pack. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. In addition, the pickles are sufficiently processed by heat to assure preservation of the product in hermetically sealed containers. The distinguishing characteristics of the various types of fresh-pack pickles are as follows:

1. Fresh-pack dill pickles are pickles that are packed in a vinegar solution with dill flavoring.
2. Fresh-pack sweetened dill pickles are pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring.
3. Fresh-pack sweetened dill relish consists of finely cut or chopped pickles packed in a vinegar solution with suitable nutritive sweetening ingredient(s) and dill flavoring. The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 1.
4. Fresh-pack sweet pickles and fresh-pack mild sweet pickles are pickles that are packed in a vinegar solution with nutritive sweetening ingredient(s).
5. Fresh-pack sweet pickle relish and fresh-pack mild sweet pickle relish consist of finely cut or chopped pickles that are packed in a vinegar solution with suitable nutritive sweetening ingredient(s). The relish may contain other finely cut or chopped vegetable ingredients as listed in Table 1.
6. Fresh-pack hamburger relish consists of relish style pickles and other chopped or finely cut vegetable ingredients as listed in Table 1 with tomato product added.
7. Fresh-pack mustard relish consists of sweet pickle relish with mustard and other chopped or finely cut vegetable ingredients as listed in Table 1.
8. Fresh-pack dill relish consists of relish style pickles containing dill flavoring and other chopped or finely cut vegetable ingredients as listed in Table 1.
9. Fresh-pack dietetic pickles are pickles that are packed with or without the addition of sweetening ingredient(s), salt (NaCl), or other suitable ingredient(s) as declared and permitted under FDA regulations.

3. Refrigerated Type

The pickles are prepared from fresh cucumbers and are packed in a vinegar solution with other ingredients to produce the fresh crisp characteristic of the refrigerated type. The pickles are preserved by acidification to maintain an equilibrated pH of 4.6 or below. They are stored, distributed, and displayed under refrigeration and may or may not contain one or more chemical preservatives. The various types of refrigerated pickles are the same as the types listed for the fresh-pack type with respect to ingredients except that they conform to the requirements for the refrigerated type.

E. Sizes of Whole Pickles

Sizes of whole pickles are based on the diameter and the relationship of the diameter to the count per gallon. Size designations, applicable counts, and diameters are outlined in Table 2. The diameter of a whole cucumber is the shortest diameter at the greatest circumference measured at right angles to the longitudinal axis of the cucumber.

Table 2 Sizes of Processed Whole Pickles Approximate Counts

		Glass			Metal	
Word designation	Diameter	1 qt.	1/2 gal	1 gal	No. 18	No. 12 (1 gal)
Midget	19 mm (0.75 in) or less	67 or more	135 or more	270 or more	202 or more	270 or more
Small gherkin	Up to 2.4 cm (0.94 in)	33–66	67–134	135–269	101–201	135–269
Large gherkin	Up to 2.7 cm (1.06 in)	16–32	32–66	65–134	48–100	65–134
Small	Over 2.7 cm (1.06 in) but not over 3.5 cm (1.38 in)	10–15	20–31	40–64	30–47	40–64
Medium	Over 3.5 cm (1.38 in) but not over 3.8 cm (1.50 in)	6–9	13–19	26–39	19–29	26–39
Large	Over 3.8 cm (1.50 in) but not over 4.4 cm (1.73 in)	4–5	9–13	18–25	13–18	18–25
Extra large	Over 4.4 cm (1.73 in)	2–3	5–8	12–17	9–12	12–17

F. Definitions of Terms

For an interpretation of this standard, some definitions of terms are given here.

Analytical definitions refer to analytical laboratory requirements.

1. Acid means total acidity calculated as acetic acid in accordance with the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).
2. Brix value (Brix) means the percent sugar, by weight, corrected to 20°C (68°F), as determined with a sugar scale Brix hydrometer or other instrument that gives equivalent results.
3. Degrees Baumé means the density of the packing medium determined with a Baumé hydrometer (modulus 145) corrected to 20°C (68°F).
4. Equalization means the natural (osmotic) or simulated blending between the soluble solids of the pickle ingredient and the packing medium.

   Natural equalization means equalization brought about after a period of time has elapsed after processing as follows. Sweetened pickles are considered to be equalized 15 days or more after processing. If the pickles have been sweetened in a tank prior to packing, the pickles will be considered equalized 15 days after the sweetening process began. Sour and dill pickles are considered to be equalized 10 days or more after processing.

   Simulated equalization means a method of simulating equalization by comminuting the finished product in a mechanical blender, filtering the suspended material from the comminuted mixture, and making the required tests on the filtrate.
5. Total chlorides or salt means the salt content expressed as grams NaCl (sodium chloride) per 100 milliliters packing medium; except that total chlorides in mustard pickles and chow is determined and expressed in grams NaCl per 100 grams of product.

Blemished means any unit that is affected by discoloration, pathological injury, insect injury, or similar causes to the extent that the appearance or edibility of the product is adversely affected.

1. Slightly - those blemishes which detract only slightly from the appearance of the unit;
2. Seriously - those blemishes which strongly detract from the appearance or edibility of the unit.

Color

1. Good color in the cured type means that the typical skin color of the pickles ranges from a translucent light green to dark green and is practically free from bleached areas. Not more than 10 percent, by weight, of the pickles may vary markedly from such typical color. In mixed pickles, chow chow pickles, and pickle relish, all of the ingredients possess a practically uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
2. Good color in fresh-pack and refrigerated types means the typical skin color of the pickles ranges from an opaque yellow-green to green. Not more than 15 percent, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
3. Reasonably good color in the cured type means that the typical skin color of the pickles ranges from light green to dark green and is reasonably free from bleached areas. Not more than 25 percent, by weight, of the pickles may vary markedly from such typical color. In mixed pickles chow chow pickles, and pickle relish, all of the ingredients possess a reasonably uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
4. Reasonably good color in fresh-pack and refrigerated types means that the typical skin color of the pickles ranges from light yellow-green to green. Not more than 30 percent, by weight, of the pickles may vary markedly from such typical color. In pickle relish, all of the ingredients possess a good, fairly uniform color typical for the respective ingredient. The pickles and other vegetable ingredients shall be free of off-colors.
5. Poor color in all types of pickles means the pickles fail to meet the requirements for good or reasonably good color for the respective type.

Also see the definition of misshapen.

Crooked pickles mean whole pickles that are curved at an angle greater than 60 degrees as illustrated by Fig. 1.

Curved pickles mean whole pickles that are curved at an angle of 35 to 60 degrees when measured as illustrated by Fig. 2.

Diameter in whole style means the shortest diameter measured transversely to the longitudinal axis at the greatest circumference of the pickle. Diameter in crosscut style is the shortest diameter of the largest cut surface.

Defect means an imperfection such as curved, misshapen, mechanically damaged, discolored, or other imperfection that affects the appearance or edibility of the product.

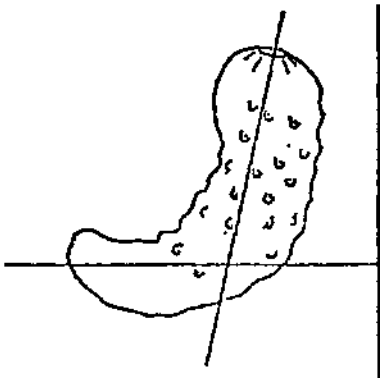

Figure 1 Crooked pickles (also see the definition of misshapen).

End cut means a pickle unit intended for crosscut (sliced crosswise) style that has only one cut surface.

Extraneous vegetable material (EVM) means any harmless vegetable material, other than stems, that is not normally part of the pickle ingredient EVM such as leaves or other vegetable material not associated with proper pickle preparation or packaging is considered a defect if it affects the appearance or edibility of the product, either

Slightly-Practically free of EVM and does not more than slightly affect the appearance or edibility

Materially—Reasonably free of EVM and does not more than materially affect the appearance or edibility.

Flavor and odor.

1. Good flavor and odor mean characteristic flavor and odor (e.g., characteristic dill flavor or the like) typical of properly processed pickles, for the type, that are free from objectionable flavor and odor of any kind.
2. Reasonably good flavor and odor mean flavor and odor that may be lacking in characteristic flavor for the type but are free from objectionable flavor and odor.
3. Poor flavor and odor mean flavor and odor that fail to meet the requirements for good or reasonably good flavor and odor.

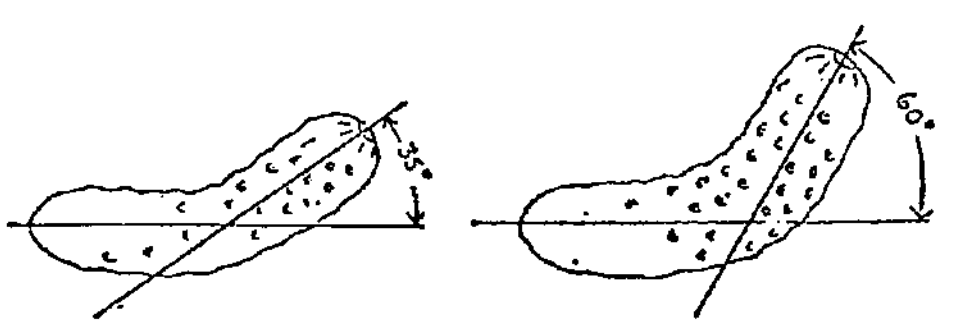

Figure 2 Curved pickles.

Length in sliced lengthwise style means the longest straight measurement at the approximate longitudinal axis.

Mechanical damage refers to crushed or broken units that affect the appearance of the units. In relish, mechanical damage refers to units which are poorly cut and have a ragged or torn appearance.

Misshapen pickles mean whole pickles that are crooked or otherwise deformed (such as nubbins). Also see the definition for crooked pickles.

A nubbin is a misshapen pickle that is not cylindrical in form, is short and stubby, or is not well developed.

Texture means the firmness, crispness, and condition of the pickles and any other vegetable ingredient(s) and freedom from large seeds, detached seeds, and tough skins that may be present. The following terms also relate to texture:

1. Hollow centers in whole style, mean that the pickles, when cut transversely to the longitudinal axis, are missing 1/3 or more of the seed cavity.
2. Soft shriveled, and slippery units refer to pickles that are wrinkled, not crisp, slick, flabby, or lack firmness.
3. Good texture means that the pickle units have been properly processed and possess a texture that is firm and crisp.
4. Reasonably good texture means that the pickle units have been properly processed but lack some of the firmness and crispness that is characteristic for the style and type of pack.
5. Poor texture means that the pickle units do not meet the requirements for good or reasonably good texture.

Uniformity of size (relish style only).

1. Practically uniform in size means that the size of the units may vary moderately in size but not to the extent that the appearance or the eating quality is seriously affected.
2. Poor uniformity of size means that the units fail the requirements for practically uniform.

Unit means one whole, half, slice, or piece of pickle as applicable for the style.

Units missing 1/3 or more of the seed cavity in crosscut style mean pickles that have lost a substantial portion of the seed cavity such as a crosscut unit missing 1/3 or more of the seed cavity portion.

G. Recommended Fill of Container

The recommended fill of container is not a factor of quality for the purposes of these grades. Each container of pickles should be filled with pickle ingredient, as full as practicable, without impairment of quality. The product and packing medium should occupy not less than 90 percent of the total capacity of the container.

H. Quantity of Pickle Ingredient

The recommended minimum quantity of pickle ingredient is designated as the percentage of the declared volume of product in the container for all items except pickle relish. Minimum quantity of pickle relish is designated as a relationship of the drained weight of the

Table 3 Recommended Pickle Ingredients (All Styles Except Relish)

Type of pack	Minimum fill (volume) (%)
Cured	55
Fresh-pack	57
Refrigerated	57

pickle ingredient to the declared volume of the container. The minimum quantities recommended in Tables 3 and 4 are not factors of quality for the purposes of these grades.

The percent volume of pickle ingredient is determined for all styles, except relish, by one of the following methods in accordance with the procedures prescribed by the USDA:

1. Direct displacement (overflow-can method)
2. Displacement in a graduated cylinder
3. Measurement of pickle liquid
4. Any other method that gives equivalent results and is approved by the USDA

Drained weight/volume. The percent weight/volume (w/v) of relish, shown in Table 4, is determined as follows:

The drained weight of pickle relish of all types is determined by emptying the contents of the container upon a U.S. Standard No. 8 circular sieve of proper diameter containing 8 meshes to the inch (0.0937 inch ± 3 percent, square openings) so as to distribute the product evenly, inclining the sieve slightly to facilitate drainage, and allowing to drain for 2 minutes. The drained weight is the weight of the sieve and the pickles, less the weight of the dry sieve. A sieve 8 inches in diameter is used for 1 quart and smaller size containers, and a sieve 12 inches in diameter is used for containers larger than 1 quart in size.

I. Sample Unit Size

For all styles of pickles and types of pack, the sample unit used in analyzing the quality factors is the entire contents of the container unless otherwise specified by USDA regulations.

J. Grades

1. U.S. Grade A is the quality of pickles that meets the applicable requirements of Tables 5–11 and scores not less than 90 points.

Table 4 Recommended Drained Weight to Container Volume, Relish

Type of pack	Minimum fill (weight/volume) (%)
Cured	
Sweet	92
Other than sweet	88
Fresh-pack	
Sweet	85
Other than sweet	80

Table 5 Analytical Requirements,[a] Cured Type Pickles, All Styles

	Total acidity expressed as acetic acid g/100 mL, unless otherwise indicated, maximum	Total chlorides expressed as NaCl grams/100 mL, unless otherwise indicated, maximum	Degrees brix, minimum	Degrees bourne, minimum
Cured type, all styles	—	—	—	—
Oils (natural, genuine, or processed)	1.1	5.0	—	—
Sour, sour mixed, dill pickle relish, sour relish	2.7	5.0	—	—
Sweet whole, sweet mixed, and sweet relish	2.7	3.0	27.0	15.0
Mild sweet, mild sweet mixed, mild sweet relish	—	—	20.0	12.0
Sour mustard or sour chow chow	2.7[b]	3.0[b]	—	—
Sweet mustard or sweet chow chow	2.7[b]	3.0[b]	28.0	15.5
Fresh-back and refrigerated types, all styles	—	—	—	—
Dills and sweetened dills	1.1	4.25	—	—
Sweetened dill relish	1.1	4.25	—	—
Sweet and mild sweet relish	1.65	2.75	—	—
Sweet and mild sweet pickles	1.65	2.75	—	—
Dietetic	—	—	—	—

[a]All pickle products must have an equilibrated pH of 4.6 or below.
[b]Expressed as grams/100 grams.

2. U.S. Grade B is the quality of pickles that meets the applicable requirements of Tables 6–11 and scores not less than 80 points.
3. Substandard is the quality of pickles that fails the requirements of U.S. Grade B.

K. Factors of Quality

The grade of pickles is based on the following quality factors:

1. Analytical requirements in Table 5
2. Flavor and odor
3. Color
4. Uniformity of size
5. Defects
6. Texture

L. Requirements for Grades

See Tables 5–11.

Table 6 Quality Requirements, Whole Style Pickles

	Grade A		Grade B	
	Maximum (by count)	Score	Maximum (by count)	Score
Flavor and odor	Good	—	Reasonably good[a]	—
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]	—	18–20	—	16–17
Diameter variation	—	—	—	—
Midget and gherkin [over 8 mm (0.31 in)]	10%	—	20%	—
Small and medium [over 10 mm (0.39 in)]	10%	—	20%	—
Large and extra large [over 12 mm (0.47 in)]	10%	—	20%	—
Defects	Practically free	27–30	Reasonably Free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Curved pickles	10%	—	20%	—
Misshapen	5%	—	15%	—
Mechanical damage	10%	—	15%	—
Attached Stems [over 2.5 cm (0.98 in)]	10%	—	20%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free	—	—	—
Soft, shriveled, and slippery units	5%	—	10%	—
Hollow centers	15%	—	25%	—
Total score (minimum)	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

II. ESTABLISHMENT INSPECTION

The U.S. Food and Drug Administration has issued guidelines for the inspection of a pickles processing plant. Some of the information is provided in this chapter. The quality control officer in such a plant should use the information to implement its in-plant inspection procedure.

The information is presented in the teacher/student format for ease of reference.

1. Direct special attention to the following areas when inspecting these types of food establishments. If the establishment is producing acidified fresh-pack pickles,

Table 7 Quality Requirements, Whole Style Pickles, Mixed Sizes

	Grade A		Grade B	
	Maximum (by count)	Score	Maximum (by count)	Score
Flavor and odor	Good	—	Reasonably good[a]	—
Color	Good	18–20	Reasonably good[a]	16–17
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Curved pickles	10%	—	20%	—
Misshapen	5%	—	15%	—
Mechanical damage	10%	—	15%	—
Attached stems [over 2.5 cm (0.98 in)]	10%	—	20%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free	—	Reasonably free	—
Soft, shriveled, and slippery units	5%	—	10%	—
Hollow centers	15%	—	25%	—
Total score (minimum)[b]	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Total score is adjusted by dividing the total score by 0.80 to allow for the absence of the quality factor of uniformity of size in whole mixed sizes style.

determine if the establishment is complying with the requirements of 21 CFR 114, Acidified Foods.

2. Salt stations and salt stock tanks.
3. Insects that breed in decomposed pickles or other decaying organic matter, such as the lesser or little house fly, the latrine fly, the house fly, the cattailed maggot, and drosophila, are of major sanitary significance. Examine 25% of the tanks for insect filth.
4. "Mill run" salt may be used but workers should not walk in the salt.
5. Tanks should be skimmed daily for debris and insects, and the skimmings should be properly disposed of.
6. In newly salted stock ferments, scum growth should be removed regularly and disposed of so that insects are not attracted.

A. Pickle Products

1. Examination of Raw Materials Used in Relish

1. Obtain the usual composition of relish in percent by weight of cucumbers as well as other ingredients to help appraise the filth load found in the sample.
2. Salt stock used for relish may consist of poor quality pickles, i.e., deformed, bloated, or blemished in the absence of filth, grit, or partly/wholly rotted pickles, there is no objection to their use. Mushy pickles are caused by certain pectin-splitting enzymes during fermentation. Soft pickles may be invaded by bacteria and fungi, but it is frequently difficult to determine if any mold or bacteria are present by field examination.

Table 8 Quality Requirements, Sliced Lengthwise Style Pickles

	Grade A		Grade B	
	Maximum (by count)	Score	Maximum (by count)	Score
Flavor and odor	Good	—	Reasonably good[a]	—
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of Size[b]	—	18–20	—	16–17
Length variation [over 2.6 cm (1.02 in)]	10%	—	20%	—
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Mechanical damage	10%	—	15%	—
Attached stems [over 2.5 cm (0.98 in)]	10%	—	20%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large seeds, detached seeds, tough skins	Practically free	—	Reasonably free[a]	—
Soft, shriveled, and slippery units	5%	—	10%	—
Total score (minimum)	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

3. Examination of cucumber salt stock for relish—when whole pickles or large pieces are used, examine a representative sample of 100 units going to chopper.
4. Segregate and list objectionable pickles as follows:

Class	Number	Percent
1. with rot spots over 1/2 in.	_____	_____
2. insect infested or damaged	_____	_____
3. mushy or very soft	_____	_____

1. For class 1 pickles, make a further determination of the surface area of the rot spots by size; up to 1 inch; from 1 inch to half of the pickle; and over half of the pickle. Take close-up color photographs of objectionable pickles. Collect exhibits of pickles showing typical rot and insect damage.
2. Laboratory examination of mushy pickles for mold is necessary to establish if they are objectionable. If over 5% of the units are mushy, cut a thin cross section from each pickle. Place the slices in a quart jar with water and add 20 cc formaldehyde for later examination.

Table 9 Quality Requirements Sliced Crosswise or Crosscut Style Pickles

	Grade A		Grade B	
	Maximum (by count)	Score	Maximum (by count)	Score
Flavor and odor	Good	—	Reasonably good[a]	—
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]	—	18–20	—	16–17
Diameter [over 5.4 cm (2.13 in)]	10%	—	20%	—
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Mechanical damage	15%	—	25%	—
Broken pieces and end cuts	10%	—	15%	—
Thickness over 10 mm (0.38 in)	10%	—	15%	—
Attached stems [over 2.5 cm (0.98 in)]	10%	—	15%	—
Units missing 1/3 seed cavity	10%	—	15%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically free	—	Reasonably free[a]	—
Soft, shriveled, and slippery units	5%	—	10%	—
Total score (minimum)	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B, regardless of the total score.

3. When small pieces of salt stock cucumbers, cauliflower, and peppers are used, rot determination by count is impractical. If rotten pieces are observed, collect a separate quart of each pickled vegetable. Preserve the samples with 20 cc formaldehyde. At the same time collect a sample totaling half a gallon of finished relish.

B. Peppers

1. Check for insect larvae (maggots or larvae of the pepper weevil) in fresh and salt stock peppers and figure percent of infestation on a representative sample. Examine any fresh-pack peppers in which infested stock was used.
2. If peppers with rot are found, evaluate in the same fashion as for cucumbers.
3. Examine vinegar storage tanks for drosophila infestation and for vinegar eels.

Table 10 Quality Requirements, Cut Style Pickles

	Grade A		Grade B	
	Maximum (by count)	Score	Maximum (by count)	Score
Flavor and Odor	Good	—	Reasonably good[a]	—
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size[b]	—	18–20	—	16–17
Small pieces 5 g or less	5%	—	10%	—
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Mechanical damage	10%	—	15%	—
Attached stems over 2.5 cm (0.98 in)	10%	—	15%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically free	—	Reasonably free[a]	—
Soft, shriveled and slippery units	5%	—	10%	—
Total score (minimum)	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.
[b] Pickles that are substandard for uniformity of size cannot be graded above U.S. Grade B regardless of the total score.

4. Insect filth in sweet stock pickle—insects, particularly drosophila, are attracted to the sweetening tanks and may be found in the finished sweet pickle products.
5. Sweet brine is frequently circulated within a tank and from one tank to another dispersing insects in the circulating brine. It is sometimes difficult to estimate the number of insects and parts in such circulating brine. Close examination of the inside tank walls may reveal drosophila above the brine level. These are the best indices of infestation in a tank.

When insects are found in a sweetening tank,

1. Determine whether sweet brine in the tank is an intermediate or a finishing brine and if it is circulated within the tank or between sweetening tanks.
2. If the finishing brine is used as a packing medium, determine whether it is filtered prior to use and evaluate the filtration step.
3. If sweet stock is held in infested tanks, determine anticipated date of packing.
4. Evaluate tank covers used.
5. List quantitatively the extent of insect infestation by the collection of representative samples of filth from a definite area, e.g., square feet of the walls of the tank on the sweet stock and in a specified amount of brine from different areas of the tank if the infestation is widespread. If infestation seems to be isolated, collect specimens showing the types of insects.

Table 11 Quality Requirements of Relish

	Grade A		Grade B	
	Maximum (by weight)	Score	Maximum (by weight)	Score
Flavor and Odor	Good	—	Reasonably good	—
Color	Good	18–20	Reasonably good[a]	16–17
Uniformity of size	—	18–20	—	16–17
Overall appearance	Good	—	Reasonably good[a]	—
Defects	Practically free	27–30	Reasonably free[a]	24–26
Blemished (slightly and seriously)	15%	—	25%	—
Blemished (seriously)	5%	—	10%	—
Poorly cut	10%	—	15%	—
Loose stems over 3.0 mm (0.12 in)	10%	—	15%	—
Extraneous vegetable material (EVM)	Practically free	—	Reasonably free[a]	—
Texture	Good	27–30	Reasonably good[a]	24–26
Large objectionable seeds, detached seeds, and tough skins	Practically free	—	Reasonably free[a]	—
Soft, shriveled and slippery units	5%	—	10%	—
Total score (minimum)	90 points		80 points	

[a] Cannot be graded above U.S. Grade B, regardless of the total score.

C. Other Points of Interest

1. Grit in pickles—excessive grit is frequently found in fresh-pack pickles and in midget sweet pickles. Salt stock may occasionally contain excessive grit. If dirty cucumbers are packed, collect in-line and finished product samples.
2. Use of color and preservatives-green artificial color is sometimes used in relish without label declaration. Ascertain if the color is permitted for use and declared on the label.
3. Sorbic acid may be used in salt stock, to preven yeast growth, and in finished pickle products, as a preservative. Where sorbic acid is present in the finished product, determine if it is declared on the label.
4. Examination of warehouse stocks—examine for evidence of spoilage, particularly in fresh pack pickles which may have been inadequately pasteurized.
5. If heavy insect infestation is found, examine 24 jars of the pickle product (other than relish) most likely to contain insects by inverting jars under strong light. Collect jars containing insects as a factory sample.

D. Sample Collection

1. Bulk Salt Stock for Filth

If in barrels, collect a minimum of 12 half-gallon jars of salt with their brine, two from each of six previously unopened barrels, to make six duplicate subs. Collect 1 sub from the

top and the other sub from the bottom, if possible. If in tank cars, collect a minimum of twelve half gallon jars of salt stock and brine. If live flies are observed inside tank during sampling, note and estimate their number.

2. Finished Pickle Product—All Types

a. Filth and Grit

Quarts and smaller jars	*Minimum to collect*
Up to 100 cases in lot	24 jars
More than 100 cases	48 jars
Gallon jars	
Up to 100 cases	12 jars
More than 100 cases	24 jars

b. Undeclared Color and Chemical Additives Collect 6 quarts or 12 pints for examination.

39
Baker's Yeast

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I. INTRODUCTION

In France, the general public is often confused between baking powder, incorrectly called "chemical yeast," and baker's yeast, which is biological in origin. The availability of active dried yeast, a staple product, only adds to the confusion. The packaging is similar and contains a powder, whereas the baker retails or sells small 42-gram cubes of a semimoist product, with a plastic appearance.

The craft baker, however, knows that the bread he makes will not be as light or have the same texture and organoleptic properties with the first product. Bread is a symbolic food about which there is universal agreement, despite the different ways it is made, because a special production method is used: fermentation. Fermentation is a 5000-year-old process that is based on the renewal of a previously risen dough: the starter dough (*levain*). Since earliest antiquity, the manufacture of bread and beer went hand in hand. In the palaces of ancient Egypt, leavens were activated by collecting the foam that rose to the surface of mash tubs. Pliny the Elder, a first century Roman naturalist, writes that the bread made in Gaul and on the Iberian peninsula owed its incomparable lightness to the addition of this foam. This method, which was taken up again in the seventeenth century to make a soft bread and was much talked about, was subsequently widely used (1). When brewers in the sixteenth century opted for low-fermentation yeast, instead of high-fermentation yeast, it was not at all suitable for bread manufacture. Distiller's yeast filled the gap. It soon opened the door in Europe to a fledgling industry, which expanded, particularly in Austria in 1846 with the Mautner process, then in England in 1886, with continuous aeration of a culture medium. This was the beginning of the biotechnology era, although people were unaware of this at the time. In fact, it was only in the years between 1857 and 1863 that Louis Pasteur demonstrated the role of yeast as the microorganism responsible for fermentation. The yeast industry made a crucial advance between 1910 and 1920 when Sak, a Dane, and Hayduck from Germany developed the continuous sugar feed process in the presence of oxygen, known as "Zulaufverfahren" or fed-batch process (2).

Worldwide production of baker's yeast was about 2.8 million metric tons a year in 2003. This is the largest quantity of microorganisms produced, thanks to the enormous

technical and scientific progress this industry has been able to exploit and develop. As a result of the innovative processes it uses, the yeast industry has also supplied all the fermentation industries, which are a feature of biotechnology today: production of enzymes, amino acids, vitamins, and substances with a therapeutic use—hormones, antibiotics, vaccines.

II. YEAST: A LIVING ORGANISM MADE UP OF A SINGLE CELL

A. Biology

1. Its Place in the Living World

The yeasts found in the flora of cereals belong to different genuses: *Pichia*, *Candida*, *Saccharomyces*. The latter is the most common, comprising the species *cerevisiae* (80%), *exiguus*, *minor*, *uvarum*, *ellipsoïdus*, and *turbidans* (3).

Although the *Saccharomyces* genus is generally used in brewing, wine-making, and baking, the *cerevisiae* species does not give the best results in each of these applications. This is why *S. carlsbergensis* (which became *S. uvarum*) is used in the first instance and *S. bayanus* in the second. Within the *cerevisiae* species, some varieties have been improved by genetics, giving rise to new strains that are better suited to their specific applications. Although *Saccharomyces cerevisiae* is used throughout the world in bread-making, there are other groups of yeasts that have very specialized applications in baking (Table 1) (4–6).

Saccharomyces cerevisiae is undoubtedly the most cultivated yeast. All research and development work focuses on it because it has the cell characteristics of higher organisms, simple nutritional requirements, and a beneficial multiplication rate, although it does not multiply quite as fast as bacteria. From an economic point of view, its suitability for industrial production and wide range of applications make it unique. Strains are generally stable; they can be improved and pose no risk in terms of food safety. Some of the different genuses and species of yeast listed above for special applications cause production problems, such as flocculation or very low yields.

Yeasts as a whole are microorganisms that have a high nutritional value and are completely harmless. However, a few of them are pathogenic. This applies to some species belonging to the *Candida* genus. Yeast producers are very cautious about using some genuses whose taxonomy is uncertain. They can be classified more accurately using very delicate genetic techniques when morphological or biochemical characteristics are not sufficient.

Table 1 Yeasts for Different Baking Applications

Applications	Genus	Species
Multipurpose	*Saccharomyces*	*cerevisiae*
High-sugar doughs	*Saccharomyces*	*rosei*
	Saccharomyces	*rouxii*
Flavor enhancement	*Saccharomyces*	*delbrukii*
	Candida	*lusitaniae*
Sourdough starters	*Saccharomyces*	*exiguus*
	Torulopsis	*holmii*
	Candida	*milleri*

Source: Ref. 4.

2. The Cell: Organized in a Similar Way to Higher Life Forms

A baker's yeast preparation, through the electron scanning microscope, reveals a multitude of distinctive cells (Fig. 1), ovoid in shape. Some of them have buds or the scars that they have left. They vary in size from 6 to 8 μm. One cubic centimeter of compressed yeast with a 30% dry matter content contains about 10 billion cells. Transmission electron microscopy reveals the following ultrastructure (Fig. 2), from the outside towards the inside:

The cell wall. Composed of glucans and mannans bound to proteins, it provides physical external protection. It is completely permeable to water, minerals, and small organic molecules.

The cytoplasmic membrane is made up of glycolipids and glycoproteins. It controls the exchanges between the intracellular and extracellular media. It is characterized by a selective permeability: it allows water and some solutions to circulate while retaining the large molecules.

The cytoplasm is a colloidal substance in which a multitude of biochemical reactions take place and which contains organelles in suspension:

The nucleus contains the chromosomes (carriers of genetic information), which transmit hereditary characters and control the synthesis of proteins.

The ribosomes, sites of protein synthesis

The endoplasmic reticulum and Golgi bodies, a network of membranes involved in the secretion of proteins.

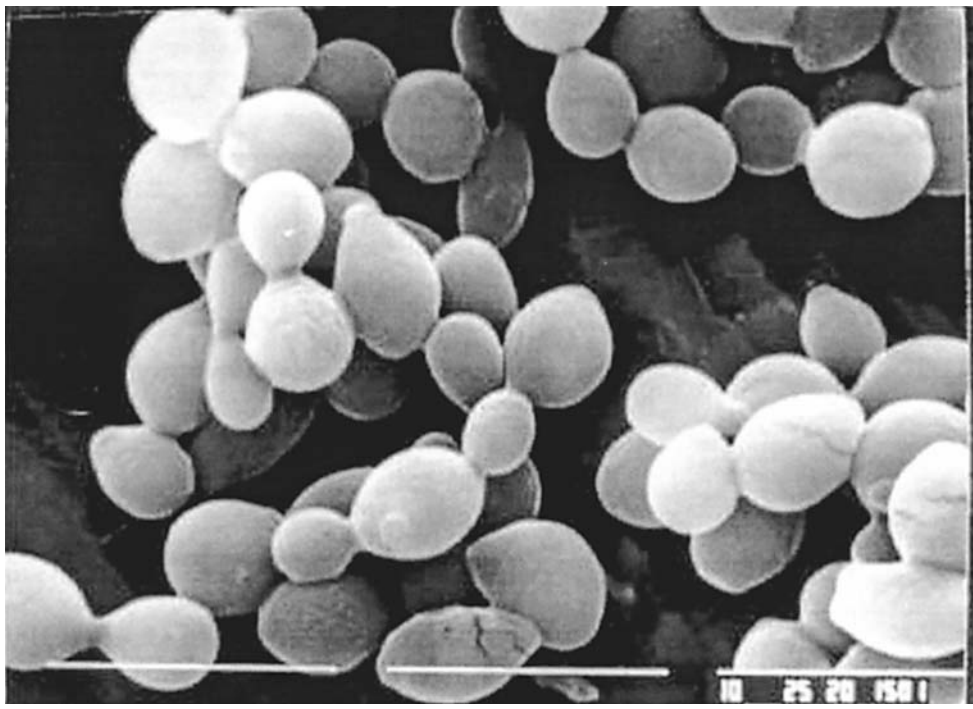

Figure 1 Electron scanning microscopy: yeast cells and buds.

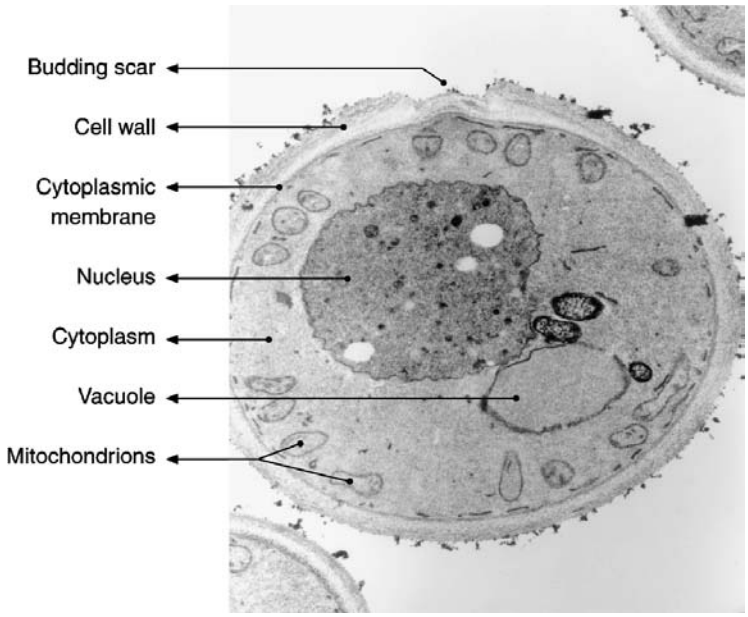

Figure 2 Transmission electron microscopy: ultrastructure of the yeast cell.

The mitochondria, energy-producing bodies in the cell in the presence of oxygen

The vacuoles, places where different storage substances are found.

B. Biochemical Composition

The composition of yeast depends on its type and the conditions in which it is stored. Table 2 gives average indicative values for samples of fresh European yeast.

The proteins contribute to a considerable degree of potential metabolic activity because they are made up of a high proportion of enzymes. The protein content is therefore directly related to fermenting power and the ability to produce biomass (see Sec. IV. C and Sec. V.B.).

The carbohydrates are mainly:

Glucans and mannans, wall constituents;

Glycogen, a storage macromolecule usually found in animal cells, which is used when there is a long-term deficiency of nutrients;

Trehalose, a disaccharide which is called up preferentially when there is a short-term deficiency. The storage of this sugar is very important whenever the yeast cell has to undergo stress, such as drying, high osmotic pressure, or freezing.

The lipids, particularly lipoproteins and phospholipids, are involved in the makeup of the cytoplasmic membrane and in maintaining its properties in the various processes used for drying active yeast.

Table 2 Average Composition of European Yeasts

Dry matter * (DM)	Percent 30.0 to 33.0			Percent
Nitrogen/DM	6.5 to 9.30			
Proteins/DM (nitrogen × 6.25)	40.6 to 58.0	of which	glutathione	0.5–1.5
Carbohydrates/DM	35.0 to 45.0	of which	glycogen	5–10
			trehalose	8–20
Cell lipids/DM	4.0 to 6.0	of which	phospholipids	1–2
Minerals/DM	5.0 to 7.5	of which	potassium	0.8–2.0
			sodium	0.01–0.2
			calcium	0.02–0.15
			magnesium	0.04–0.18
			phosphorus	0.8–1.3
			(P_2O_5)	2.0–3.0
Vitamins		of which	Thiamin (B1)	0.002–0.015
			Riboflavin (B2)	0.002–0.008
			Pyridoxine (B6)	0.002–0.006
			Niacin (PP)	0.010–0.050

The minerals—including phosphorus which is essential as it is involved in the formation of nucleic acids, molecules with a high energy potential (ATP), and membrane phospholipids.

Due to high levels of proteins in deactivated yeast, with its presence of all the essential amino acids in addition to phospholipids, minerals and vitamins, it is regarded as a top-quality food supplement.

III. BEHAVIOR OF YEAST DURING BREAD FERMENTATION

A. The Roles of Yeast in Bread-Making

1. Dough Rising

This is the most obvious phenomenon for anyone who is unfamiliar with bread manufacture. Air is incorporated into dough during mixing and the yeast is able to establish respiratory-type metabolism. A few minutes after mixing, all the oxygen that has been introduced is used up by the yeast. Consequently, due to the anaerobic conditions, yeast metabolism is geared towards fermentation. The carbon dioxide produced firstly dissolves in the free water in the dough. When it reaches saturation point, it accumulates in gaseous form, exerting internal pressure on the impermeable gluten network. The latter, which is elastic and extensible, enables the dough to rise, while the external structure is maintained. Contrary to what is generally thought, the pores in the crumb of bread have nothing to do with the distribution of yeast cells but correspond to the dilatation of the CO_2 they produce. This diffuses into the air bubbles, which are incorporated and dispersed in the dough during mixing and the various mechanical operations.

2. Acidification

The formation of carbon dioxide and organic acids results in a lowering of pH and an increase in the total titratable acidity (TTA) of the dough during fermentation, in spite of the

high buffer capacity of proteins in the flour. This acidification confirms that bread fermentation is working properly. It is often measured (pH and TTA) in routine checks in industrial bakeries using prefermentation processes as water brew, stiff, or liquid sponge.

3. Flavor Production

The alcohol formed, the lowering of pH, and the release of metabolites from secondary fermentation are directly involved or act as precursors in the development of bread taste and flavor (7–9). Long fermentation times, slightly low dough temperatures, and sensible quantities of yeast result in a bread with excellent organoleptic properties. This is on condition, of course, that the dough has not been subjected to excessive oxidation which is the result of high-speed mixing and the presence of bean or soya flour.

Particular genuses and species of yeast have metabolisms that result in bread products with very characteristic flavors.

4. Change in Dough Rheology

Apart from the physical changes dough undergoes during the various operations of mixing, dividing, rolling, or molding, its viscoelastic properties are transformed throughout the fermentation process. This is a very familiar phenomenon to the craft baker, for whom the effects of bread fermentation do not simply mean the inflation of dough (10). The dough strengthening to which bakers refer, and which is occurring during bulk fermentation, is a perfect illustration of this. It involves a reduction in gluten extensibility combined with an increase in its elastic resistance (Fig. 3).

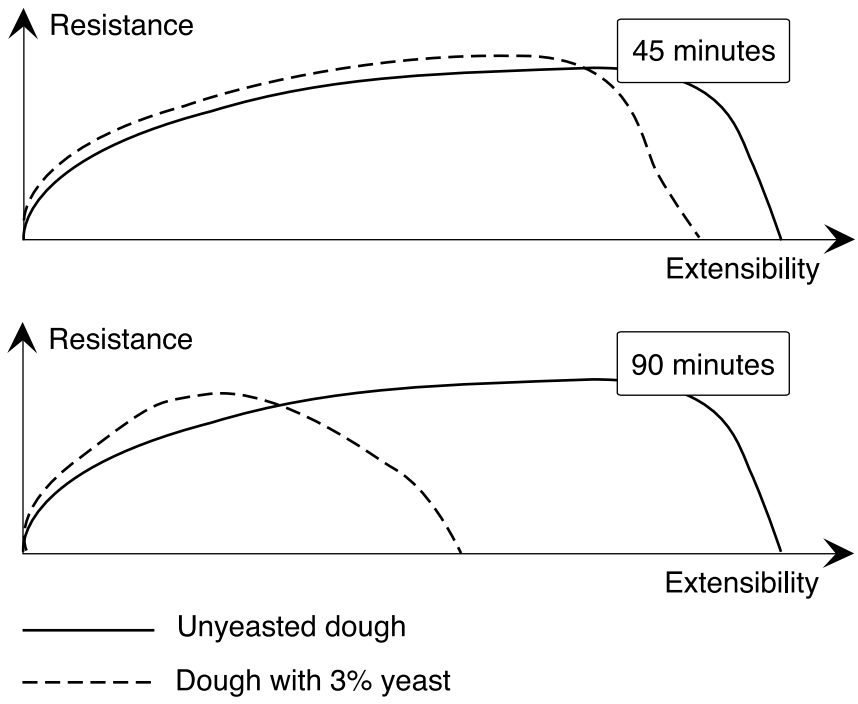

Figure 3 Effect of fermentation on dough strengthening.

The causes are known but it is difficult to find a scientific explanation for the mechanisms because of the complexity of the dough system. It involves:

First, a purely mechanical effect that is the result of gluten development; namely, its extension and organization into a three-dimensional network under gaseous pressure;

Second, the formation of physicochemical bonds, strengthening the cohesion of the gluten network. The lowering of pH, reactions with the different metabolites produced by secondary fermentation, and variations in surface tension between the different dough phases seem to play a part in these phenomena. According to K. Hoseney, the phenomenon is essentially oxidative, involving the production of oxygen peroxide by the yeast (11).

Understanding the notion of dough strength is complicated by certain factors that can cause confusion, particularly in long fermentation processes. After a long first fermentation, or the fermentation of a sponge, the dough softens, which seems to go against this idea of strength. This effect, which is real, is heightened by the under-mixing inherent in this type of dough. In fact, it is linked to the gradual hydration of gluten, and to the action of enzymes in the flour: the amylolytic activity, which contributes to the release of water previously fixed by the starch, a slight proteolysis affecting the gluten; and possibly also the reducing effect of the glutathione excreted by some types of dried yeast.

These changes in no way preclude the strengthening of dough elasticity, which takes place in the gluten and is evident during the molding operations. Too much strength will affect the external and internal characteristics of the finished product, so the baker's skill lies in knowing how to control fermentation processes to fit the characteristics of the flours and the type of mixing used. This skill is somewhat restricted by high-speed mixing methods and the use of dough conditioners. The fact remains that in reality the technology is complex, making it difficult for bakers and technicians or engineers in the world of industry or applied research to communicate. The only way forward is for the latter to "get their hands dirty" if they wish to establish a fruitful dialogue based on mutual trust.

B. Use of Fermentable Substrates: The Enzymes Involved (12)

Monosaccharides, simple sugars with six carbon atoms (such as glucose, fructose, and galactose), are preferentially used by *S. cerevisiae*. Nevertheless, the assimilation of galactose depends on the concentration of glucose, with the latter exerting catabolic repression. It is generally acknowledged that glucose and fructose can penetrate the cell by facilitated diffusion, involving phosphorylation.

Disaccharides can be assimilated after enzymatic hydrolyses.

The sucrose already present in flour or added to the ingredients is converted to glucose and fructose by invertase in the yeast. This reaction takes place in the periplasmic space between the wall and the cytoplasmic membrane. The two hexoses then diffuse into the cytoplasm where they are metabolized. Invertase acts very quickly, practically doubling the osmotic pressure in the region next to the cell.

Maltose, which mainly comes from the conversion of starch by action of the α-alpha and β-beta amylases in flour, is split into two glucose molecules by maltase, an enzyme in yeast cells. However, the maltose must have previously been carried inside the cell by maltopermease.

Not all strains of yeast have the same ability to ferment maltose. Strains used in the USA and Japan have adaptive maltopermease and maltase. The synthesis of these enzymes is catabolically repressed by glucose and induced by maltose, so the cell can only produce the two enzymes that enable it to use the maltose present in the medium after the glucose has been exhausted (13,14).

In Europe, most industrial baker's yeasts have constituent maltopermease and maltase. These strains were developed in Great Britain in the early 1960s for the Chorleywood Bread Process. They were then used on the continent where high-speed mixing was catching on. These types of strains were able to rapidly adapt to maltose in bread-making processes with no added sugar, because mechanical dough development replaced first fermentation in straight doughs, or sponge in sponge and dough processes. The risk of exhausting the fermentable sugars in the dough was thus to be eliminated by systematically supplementing flours with malt or α-amylases.

There are also small quantities of tri- and polysaccharides, which are fermentable to varying degrees.

C. Fermentative Profiles

This ability to ferment maltose is easily demonstrated by the Rheofermentometer, an equipment for recording the variations in a yeast's rate of fermentation as a function of time (for example, CO_2 released per minute). The dough used must have no sugar added to the flour (so-called normal dough).

Depending on the quantity of yeast, its fermentative capacity, or the fermentation temperature, a "depression of adaptation to maltose" varying in size can be seen on the curve. This depression in fermentation speed is linked to the exhaustion of directly fermentable sugars already present. If the yeast contains constituent maltase and maltopermease, the depression is much less pronounced, or nonexistent, depending on the above-mentioned conditions.

If there is added sugar (sucrose, glucose/fructose syrup), the depression disappears as yeast preferentially uses these substrates (Figs. 4 and 5).

D. Influence of Various Factors on Fermentative Activity

For a strain with a given biochemical composition, the conditions of the medium affect the rate of fermentation of yeast. The effects must be taken into account in baking, not only for the sake of productivity and economics but also for technological reasons. For example, there are the problems of variations in dough density when passed through a volumetric divider, or variations in dough strengthening between the start and end of molding (the larger the dough mass being divided, the greater the difficulty).

Although numerous factors have a bearing on the fermentative activity of yeast, we should remember that the baker judges this activity by the rising of the dough. This is the result of the force exerted by the increase in internal pressure (impermeability + CO_2 production) and the resistance of the dough to deformation. Extensibility, elastic resistance, permeability, viscosity of dough (cold or warm, soft or firm) are so many parameters that

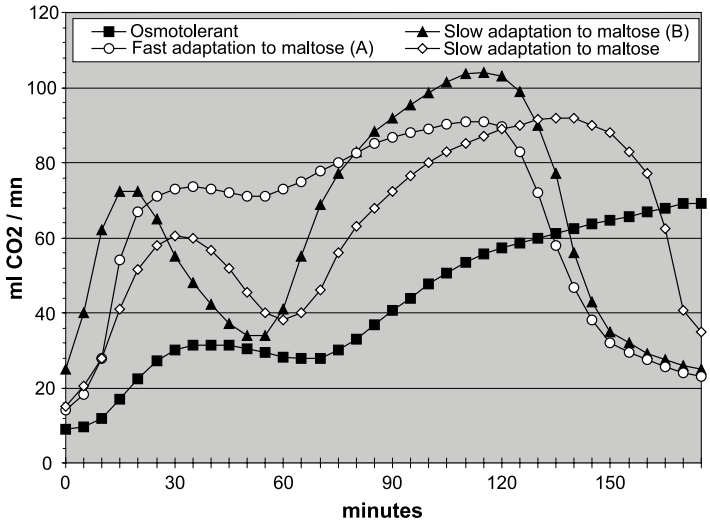

Figure 4 Strains: fermenting profiles. 0% sucrose, yeast dosage 3%, T = 27°C.

affect the rate of dough rising, regardless of the activity of the yeast itself. This is clearly demonstrated by observing a dough piece as it rises in a mold, in a fermentation chamber at 43°C and 85% relative humidity. The dough "moves" slowly at first, but its volume grows more and more quickly. Over a final fermentation period of 1 hour, the last 5 minutes are crucial as, in that time, there is a spectacular increase in the height of dough in the mold. This is related, among other things, to a reduction in internal pressure as the dough swells, as can be seen on the alveograph curve.

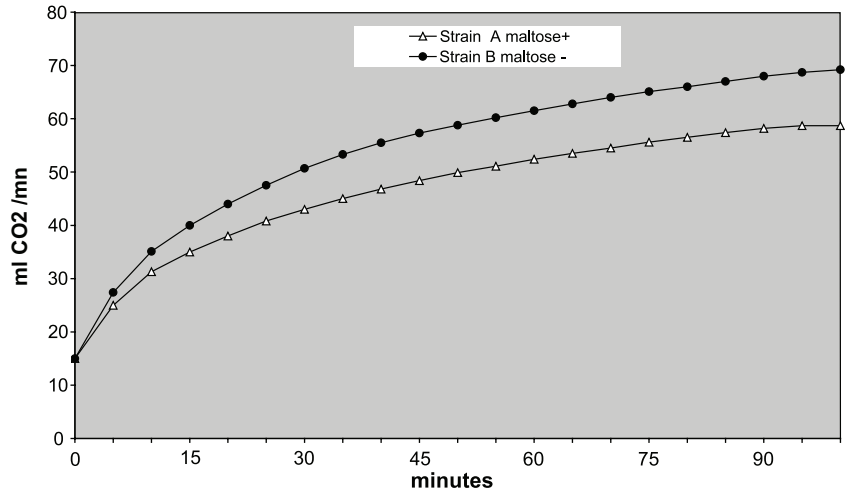

Figure 5 Fermenting profiles of strains A and B. 7% sucrose, yeast dosage 3%, T = 43°C.

1. Effect of Temperature

Enzyme activity in the yeast depends on the temperature of the medium. Within a range of 20 to 40°C, an increase in dough temperature of 1°C results in an increase in fermentation speed of 8–12% according to the type of yeast (Fig. 6). In production conditions, therefore, it is vital to check and control dough temperatures very accurately at the end of mixing, and particularly in the laboratory when comparative studies are being carried out.

This explains the variations of dough and fermentation temperatures required for various applications. Cooler doughs are needed for baguette production (22–24°C) in order to restrict dough strength, which would hinder molding, and to encourage certain flavors to develop. When working with raw frozen doughs, temperatures of between 18 and 20°C restrict the startup of yeast activity. At 4°C, fermentation is held up which retarded dough process to take place. Final fermentation temperatures of 30–35°C are used for tinned sandwich bread in France, but the general average in the United States is 48°C, which increases productivity but at the expense of the overall quality of the finished product. In the United Kingdom, fermentation temperatures of 55°C for soft bun manufacture are quite common, and can be as much as 75°C in the case of industrially produced pizza crusts, all at relative humidity of 95%. It should be noted that yeast is very quickly killed when the internal dough temperature exceeds 55°C.

2. Effect of Osmotic Pressure

The semipermeable cytoplasmic membrane of the cell delimits two compartments. When there is a difference in concentration between the medium inside and outside the cell, water gradually moves from the less concentrated medium to the more concentrated one so that equilibrium can be established. This transfer is influenced by a force that exerts a virtual pressure linked to the semipermeability of the membrane. This movement of water can be prevented by exerting hydrostatic pressure. The difference in pressure between the two media defines the osmotic pressure. This is in proportion to the number of dissolved particles per unit of volume. A solution containing 1 mmole/L of sodium chloride (58 mg/L) will release

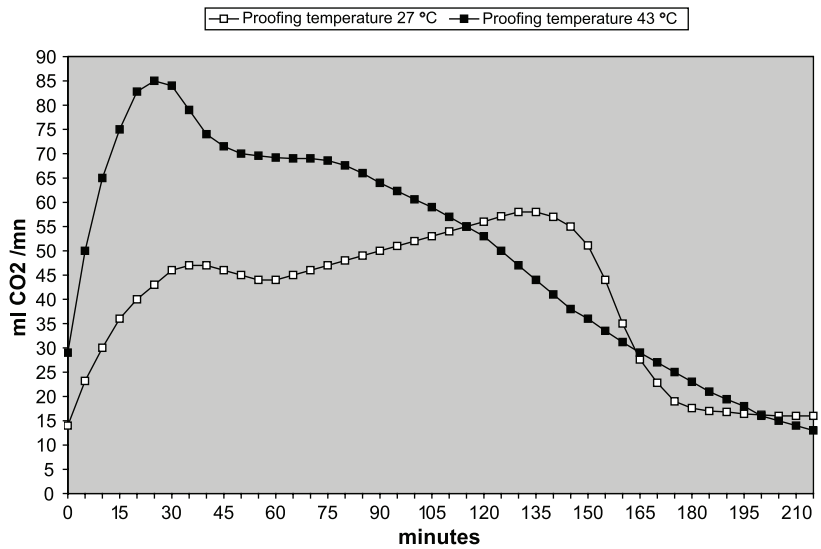

Figure 6 Effect of proofing temperature on fermentation rate. 0% sucrose, standard yeast 3%.

two ions per molecule and the osmotic pressure can be twice that of a glucose solution also containing 1 mmole/L (180 mg/L).

In a dough, osmotic pressure mainly depends on the proportions of sugar and salt used. It can be very high, as much as 35 to 100 atmospheres. This explains the slow-down in the activity of yeast in which metabolism cannot take place normally because water has leaked outside the cell and fewer fermentable sugars can enter. In bread-making, sugar, often added with fat, is responsible for a reduction in dough consistency. It then becomes necessary to decrease dough hydration, which accentuates the concentration phenomenon in the medium. The baker compensates for the drop in fermentative activity by increasing dough and fermentation temperatures, reducing the quantity of salt and increasing the amount of yeast. The Rheofermentometer shows the fermentation rate of yeast with different levels of sucrose, or salt (with or without calcium propionate added in the dough) (Figs. 7, 8). For a standard French yeast, there is a low level of sugar stimulating fermentation and prolonging it; from 5% of sugar upwards, the rate of fermentation is slowed down and tends to decrease. The rapid accumulation of alcohol in the medium, to which the yeast is very sensitive, could be the reason for this. The higher the fermentation temperature, the more obvious this phenomenon is.

There are some bread-making processes in which the concentration of sugars is such that the methods described above cannot guarantee the dough rising within a reasonable time using ordinary yeasts. This has led to the development of osmotolerant strains (Fig. 9) which are used in France (Vendée brioche); the United States of America (Danish pastries, sweet doughs, Hawaiian sweet bread), Indonesia (roti mani), Japan (kashipan) and so forth, where the levels of sugar vary from 20 to 50% based on flour weight. The osmotolerance of these strains is associated with various characteristics, including:

A low level of invertase so that sucrose is gradually converted to glucose and fructose, resulting in fewer dissolved molecules at a given moment. Obviously, this ability does not apply to sugars such as glucose/fructose syrups.

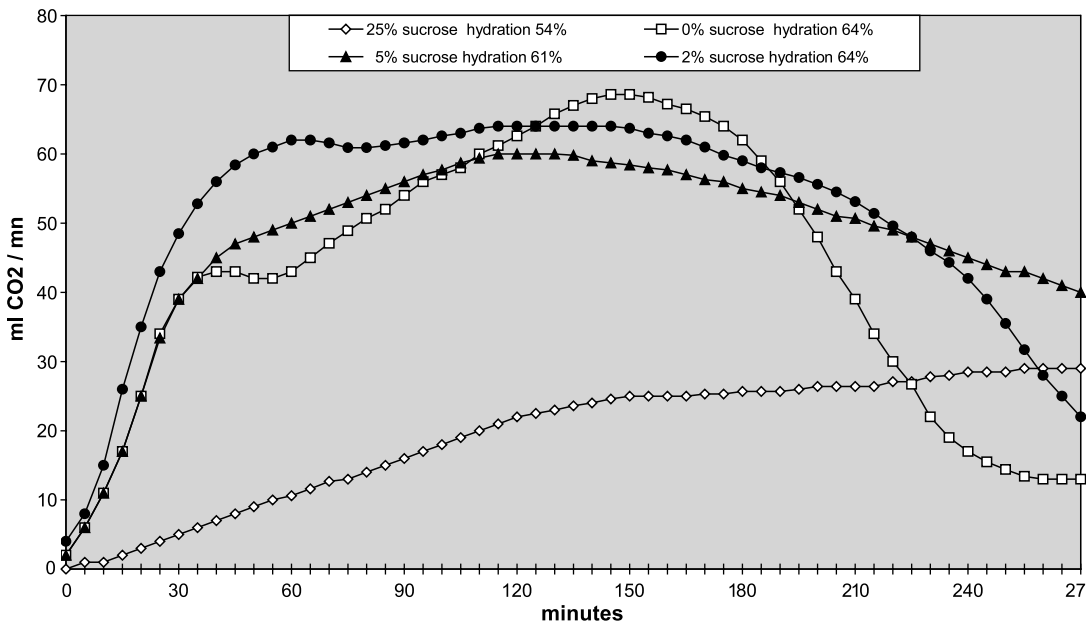

Figure 7 Effect of sucrose dosage on fermentation rate. Standard yeast 3%. T = 27°C.

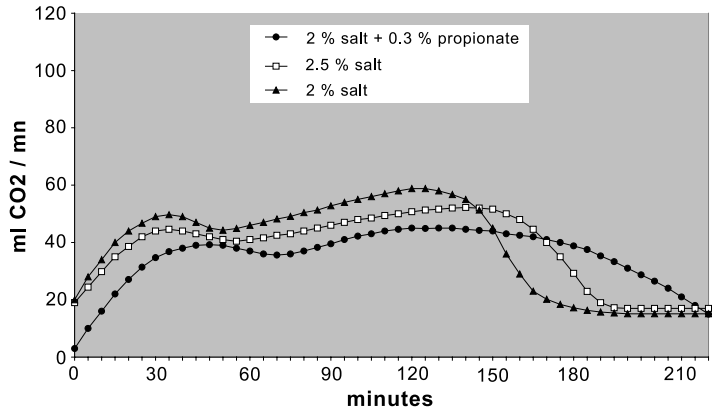

Figure 8 Effect of salt and calcium propionate on fermentation rate. 0% sucrose, standard strain 3%. T = 27°C.

The capacity to synthesize molecules such as glycerol or trehalose, so that the exit of water from a cell is counterbalanced when the concentration of the medium outside the cell is too high.

The propagation conditions on which, regardless of strain, the osmotolerance of a yeast depends.

3. Effect of Yeast Dosage

At a given temperature, the rate of fermentation depends on the quantity of yeast used (Fig. 10). We can see a depression in the curves in the first hour because the sugars already present are being used up more quickly.

If no sugar is added, the quantity of maltose resulting from amylolysis is a limiting factor. When the amount of yeast is too high, a flour with low levels of damaged starch or

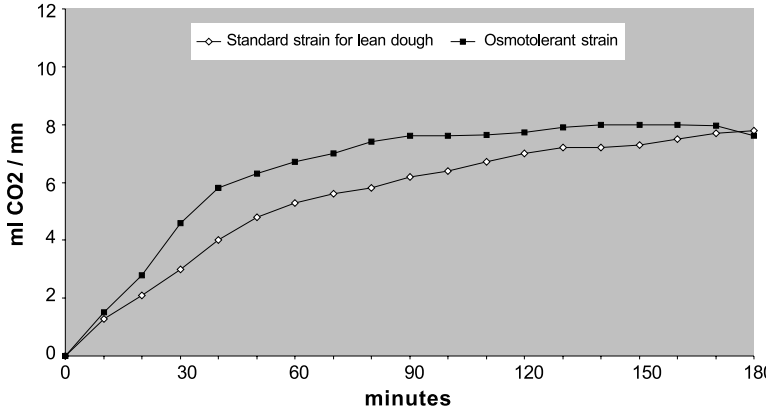

Figure 9 Behavior of different strains in sweet doughs. 10% sucrose, yeast 3%, T = 27°C.

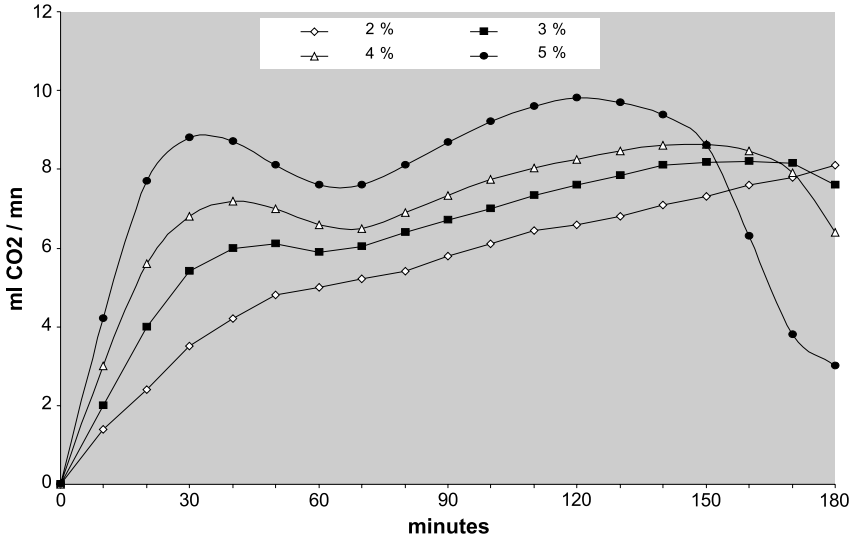

Figure 10 Effect of yeast dosage. 0% sucrose, standard strain, T = 27°C.

α-amylases can be exhausted before proofing is completed. Adding fungal α-amylases at a level of 40,000 SKB units per 100 kg of flour prolongs fermentation by about 1 hr in standard bread-making conditions (Fig. 11).

4. Effect of pH

The internal pH of a yeast cell is of the order of 5.6 to 5.8 and varies very little. The enzyme systems involved in the metabolism of *S. cerevisiae* are intracellular as a whole, which explains why yeast is very tolerant to variations in the pH of a medium, within the range of 2 and 8.

At pH of between 4 and 6, the fermentative activity of yeast is at its optimum. These conditions are found in most bread-making processes with yeast where the pH at the end of final fermentation is of the order of 5.2 ± 0.2. This pH can be lower when using sourdough

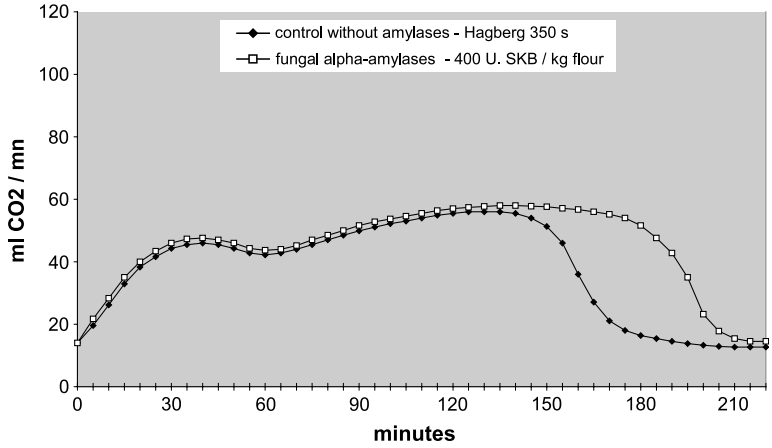

Figure 11 Effect of fungal α-amylases on fermentation rates. 0% sucrose, yeast 2%, T = 27°C.

Table 3 Changes in Dough Acidity at Different Stages of Fermentation

	pH	TTA
American sandwich bread		
Sponge (70% flour)	4.8	4.6
Dough at start of proofing	5.2	4.0
Dough at end of proofing	5.0	5.3
American sandwich bread		
Liquid preferment (20% flour)	4.5	12
Liquid preferment (50% flour)	4.8	10
French bread—straight dough		
Dough at end of mixing	6.1	1
Dough at end of proofing (2 1/2 hr fermentation)	5.8	1.5
French bread—30% sourdough		
Dough at end of mixing	5.3	2.0
Dough at end of proofing (6 hr fermentation)	4.2	7.0

starters (II, § III. 1), deactivated sourdoughs, or organic acids, particularly for rye bread manufacture. Unlike glucose, the fermentation of maltose is affected by pH of less than 4.

The actual acidity of a dough cannot be assessed by pH alone due to the high buffer capacity of flour proteins. It is often associated with titratable total acidity which is an arbitrary notion often used in the United States (TTA) and Germany (Säuergrad). In the first case, it corresponds to the quantity of 0.1 N sodium hydroxide (4 g/L) needed to neutralize a ground mixture of 15 g of dough in 100 mL of distilled water to pH 6.6. In the second, the pH is 8.5. This method enables plant bakers to control the consistency of their fermentation processes (Table 3).

Volatile organic acids and their salts inhibit fermentative activity in their nondissociated form. A low pH, which shifts the balance of dissociation toward the nondissociated form, means that the acetic acid or propionates used as antimold agents will have a much more detrimental effect. The critical zone falls right inside the range of pH (4 to 5) found at the end of fermentation in most types of bread-making methods (Table 4).

5. Inhibiting Ions

In the cases, fortunately rare, where bread fermentation is inhibited, water is often responsible as a carrier of certain ions that are toxic to yeast. Cu^{2+} from the copper in old pipes, quaternary ammoniums in antiseptic products from poorly rinsed equipment and excess chloride (Cl^{-}) in city water supplies have often been blamed.

Table 4 Dissociation of Acetic and Propionic Acids at Different pH Values

	Nondissociated fraction, %					
	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7
Acetic acid	99.8	98.2	84.7	35.7	5.3	0.6
Propionic acid	100	99	88	42	6.7	0.7

IV. INDUSTRIAL PRODUCTION OF YEAST (13–17)

In 2003, an estimated 2.8 million metric ton of baker's yeast, expressed as compressed yeast, was produced throughout the world. Half was produced on the continent of Europe, with the other half equally divided between the Americas and the rest of the world. Regardless of their physical forms (compressed, dried, etc.), the yeasts produced are not the same in the various regions of the globe, because manufacturers have to adapt them to the local bread-making conditions. However, they have one problem in common; ensuring that the fermenting capacity of the product they supply is consistent and stable. This is achieved by controlling the quality of raw materials, production technology and logistics.

A. Raw Materials

The raw materials used must satisfy the nutritional requirements necessary for the growth and multiplication of yeast cells.

Molasses is the substrate of choice, both economically and technically. Viscous, highly colored liquids, their composition varies according to the sugar-making process from which they derive, and the quality of the sugar-beet or cane harvest; 77–82% of dry matter essentially provides sucrose as a source of carbon (45 to 55%), minerals, trace elements, and vitamins. However, they supply very little nitrogen for protein synthesis (the betaine they contain cannot be assimilated) and not enough minerals such as phosphorus, magnesium, and often zinc. The problems they cause are rarely related to any deficiencies but are more likely to be due to the presence of elements that are toxic to yeast. These may be substances that derive from farming methods or sugar-producing processes: fungicides, quaternary ammonium, sulfites, but also an excess of minerals (Na^{2+}), short-chain fatty acids, and a great many trace elements.

In order to homogenize the composition of the nutrient medium and reduce the risks of toxicity, all batches are analyzed and tested before they are mixed (up to 10 or 12 different sources).

After an initial filtration to remove coarse foreign elements, the molasses is diluted and heated to reduce the viscosity. Clarifiers continuously remove the fibers and colloids by centrifugation. The next is flash pasteurization: the molasses is heated to 130°C under pressure for a very short time. This treatment removes vegetative forms of microbial contaminants as well as *Clostridium* and *Bacillus* spores, without caramelizing or degrading the sugars. After it has cooled down on plate exchangers, the molasses is stored in a buffer tank, ready for use.

Cane molasses, which contains a lot of fibers and colloids, is difficult to process but has a very high nutrient content. When it is mixed at a level of 20% with sugar beet molasses, biotin does not need to be added.

The minerals that are added as nutrients (phosphoric acid, ammonium salts, ammoniac) or to control pH (sulfuric acid, soda) comply with food standards, in particular for heavy metal content. The water for diluting the medium is potable and chlorinated to avoid any contamination.

B. Storage and Protection of Strains

Thousands of strains have resulted from research studies but less than about 10 are used in large-scale industrial production. Manufacturers store them in their own laboratories but also, as a precautionary measure, in public collection centers. With the development of

identification methods, the claims of patent applications dealing with the creation and improvement of strains can be described more accurately. The methods of storing strains vary; for example, −80°C on a glycerol medium with subcultures made after 1 to 5 years, or 4°C on a gelose medium and subcultures after 1 to 3 months.

C. Propagation

How yeast cells multiply (wrongly called fermentation) is of major importance in carrying out the yeast manufacturer's two objectives.

The first relates to quality. A specific strain must satisfy many criteria, which are as follows

The best fermentative power in a specific application ("normal," sweet, or acid doughs) or fermentation kinetics that are most suited to a given bread-making method (sponge and dough, Chorleywood bread process, etc.)
The nature of the finished product: compressed yeast, rehydratable dry yeast or instant yeast
The stability of fermenting power

The second objective has to do with economics. The manufacturer must achieve the quality he seeks for the best price. The result is a compromise between:

Yields (% of yeast obtained in proportion to the substrate used)
Productivity (rate of multiplication and optimum use of tank equipment)

It is evident, therefore, that there are many complex and sometimes contradictory obstacles. To untangle the knot, engineers and researchers rely on data on the biochemistry and physiology of cells. Although attempts at modeling have been made in order to optimize production, experience and a certain degree of pragmatism are still called for.

Yeast is propagated in sequences during which cells multiply under different conditions. Multiplication has two aims, which follow on from each other:

The first is to massively increase the cell population so that a final so-called commercial generation can be seeded.
The second is to multiply the yeast so that its biochemical composition and physiological condition meet the requirements of producer and user.

The number and method of these stages can differ noticeably from manufacturer to manufacturer, but the principle is the same. In each stage, enough yeast is produced to inoculate the next stage (Fig. 12). This is carried out using a series of containers, from lab to plant, whose contents must be managed extremely well when the industrial stage is reached.

The third industrial generation (commercial generation) must finish with a maturing phase, the aim of which is to ensure the yeast is stable. It means making the yeast, at the end of the manufacturing process, synthesize as many stock sugars as possible (trehalose and glycogen) and reducing the rate of budding to very low levels. In order to do this, the yeast is deprived of nitrogen, supplying it with molasses only and the temperature is slightly increased above the 32 °C ± 2, which is the average temperature during production.

Next comes the separation of yeast cells from the wort. This operation takes place continuously using centrifugal equipment where the yeast is washed and a cream is obtained (i.e., a suspension of cells in water, at a dry matter concentration of between 15 and 20%). This figure is equivalent to about 50% of cells, in volume. Above 23% dry matter, the suspension becomes too viscous and cannot be pumped. This cream is cooled down to 4°C in

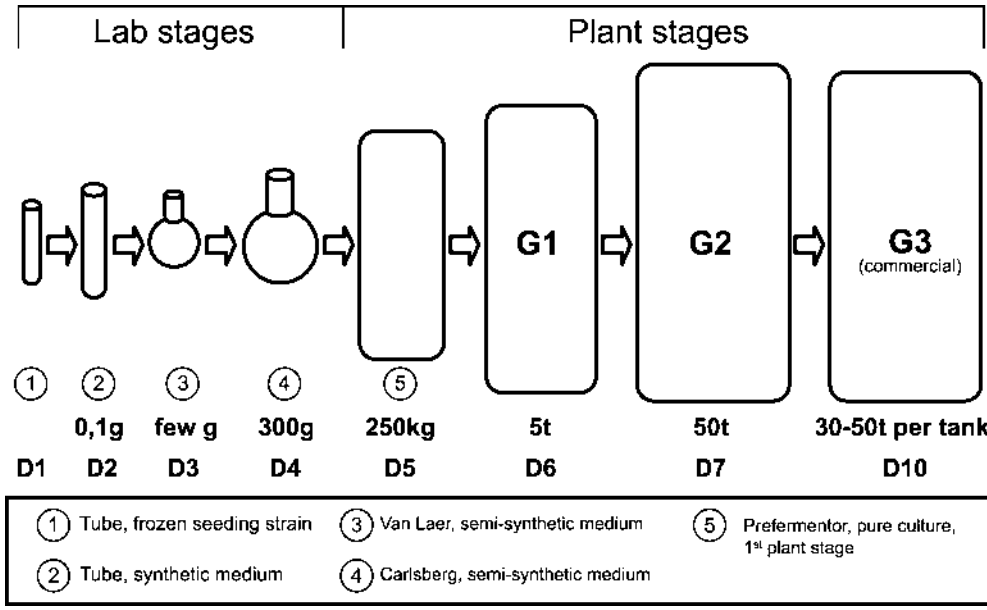

Figure 12 Yeast propagation stages. D = day.

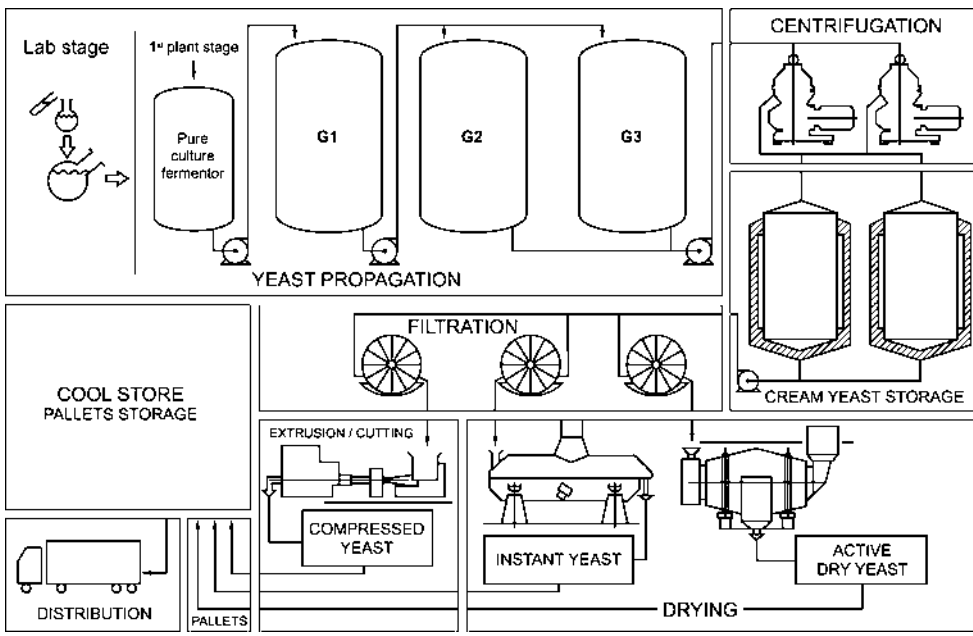

Figure 13 Yeast manufacture flow chart.

a plate exchanger and kept at this temperature in storage tanks. About 400 metric tons of yeast (compressed yeast equivalent) will be finally collected 10 days after the first test tube was cultured in the laboratory. The principle of yeast manufacture is summarized in Fig. 13).

French yeast producers, who are concerned about environmental regulations, have concentrated on investing in methods of pollution control. After the yeast cream has been separated, there is a great deal of effluent that cannot be discharged, as it is, into the environment. The most diluted effluent is treated by aerobic or anaerobic biological methods. The must from which the yeast has been removed, and which still contains nonassimilated organic nitrogen (betaine) and mineral salts rich in potassium sulfate, is very highly concentrated by evaporation and inverse osmosis. The vinasse obtained is used as a fertilizer, spread on directly as manure or in the form of potassium extracts after crystallization, decantation, and drying. It can also be used in animal feed after treatment to reduce the potassium content. Pollution control treatments have a considerable effect on production costs.

V. COMMERCIAL YEASTS

A. Strains

The strains used in France and in Europe as a whole are of the "rapid adaptation to maltose" type. They were developed in the early 1960s in response to straight bread-making methods that had appeared as a result of high-speed mixing used in bread-making with no added sugar. Because of their poor performance in very sweet doughs (over 15% of sucrose / flour), osmotolerant strains already used for the large export markets were introduced to the French market at the end of the 1980s. The difficulty bakeries had in using several qualities of yeast resulted in research work into strains with a wide range of applications (Table 5).

B. Biochemical Compositions Adapted for Applications

The need to increase the rate of fermentation, particularly in an industrial environment, is met by yeasts with a high nitrogen, and therefore protein, content. The major drawback of these yeasts is their instability, which is due to higher enzyme activity and a storage sugar

Table 5 Comparison of Commercial Strains and Wide-Range Laboratory Strains

Type of yeast and strain	Fermenting power lean dough (base 100)	Fermenting power 25% sweet dough (base 100)	Invertase activity (Ui)[a]
Commercial—rapid adaptation to maltose	100	60	60–200
Commercial—osmotolerant	50	100	5–10
Lab—hybrid, wide range	85	105	<5
Lab—genetic engineering wide range	90	100	<10

[a] 1 Unit invertase = 1 μmole of reducing sugar released/5 min/mg of yeast DM.
Source: Ref. 6.

content that is proportionally lower. In the case of compressed yeast, as has been mentioned earlier, the increase in protein level calls for a decrease in DM content so that the product maintains its physical cohesion. The stability of its fermentative activity is affected by this, which means that low temperatures must be maintained. The higher the protein level, the more rigorously these low temperatures need to be observed. An increase in temperature accelerates the consumption of trehalose, then glycogen to cover the needs of energy metabolism. When these carbohydrates are exhausted, the yeast attacks its own proteins. This autolysis is responsible for the degradation of yeast activity and its physical appearance (Table 6).The nitrogen content of the various types of dried yeast is restricted to the maximum level that is unlikely to be detrimental to them during drying.

C. Controls on the Finished Product

A yeast, regardless of its form, must comply with several criteria before it can be marketed:

Physical and organoleptic: Consistency and friability in the case of compressed yeast, shape and size of particles in the case of dried yeast, color and smell.

Biochemical: Dry matter, nitrogen, and phosphorus composition; stocks carbohydrates.

Bacteriological: Appearance of cells, budding rate; most attention is given to identifying and counting foreign microflora.

Fermenting power: Most of the methods used measure the release of carbon dioxide that is produced over a given time, by fermentation of a medium with known characteristics. Mostly, it is a wheat flour dough whose consistency varies from liquid to very firm. Various levels of sugar can also be used to evaluate the behavior of yeast at increasing osmotic pressure (18).

Suitability of yeast for storage: The stability of the fermenting power of various types of yeast can be evaluated in storage tests, at temperatures of between 20 and 43°C. There are correlations between the results of these accelerated tests and those obtained in real time, at temperatures found in different markets.

Bread-making tests: For specific applications, laboratory fermentation tests, although useful, do not supply enough information about the overall yeast activity (see Sec III.A.). Bread-making tests can result in a better understanding of the effect of yeast on dough rheology, on dough behavior during proofing and baking, and finally on its effect on bread properties: color, structure, crumb texture, and flavor. Monitoring the dough in a Rheofermentometer at the same time can provide a wealth of information.

Table 6 Change in the Fermenting Power of Various Types of Yeast in Accelerated Storage Tests

Yeast quality	DM, %	Nitrogen/DM,%	Fermenting power, lean dough (base 100)	Loss after storage, 7 days / 26°C
Standard	≃ 33	7.0	100	−10%
Rapid	≃ 32	7.3	110	−25%
Ultra rapid	≃ 31	8.2	125	−50%
High activity	≃ 29	9.3	165	Autolysed

D. Commercial Forms and Methods of Use

The different commercial forms of baker's yeast can be discussed in the order in which they appear in the manufacturing process, which corresponds to a continuity in their historical development. And although the 1990s saw a sudden demand for liquid yeast in industry, this was no revolutionary product unless we are thinking of the mechanical meaning of the term revolution: a return to the point of origin!

1. Liquid Yeast

Until 1825, when compressed yeast was introduced by Tebbenhof, yeast was sold in liquid form (2) . The return to this form corresponds to the demand from plant bakeries. In general use in Australia, liquid yeast is used much less widely in the United States and Canada and has its followers in the United Kingdom and Ireland.

Advantages:

Dosage of the yeast can be automated, as it is for the other raw materials, resulting in improved control and stock management.

No handling and no packaging materials to dispose of.

Even dispersion in the dough during high-speed mixing.

Yeast activity is standardized: the producer controls fermenting power using a substitution rate: for example, 1.5 L of cream per 1 kg of compressed yeast.

Stability is noticeably improved compared with other forms, because the cream is cooled immediately on a plate exchanger. This stability is ensured by monitoring the storage temperature (Table 7). However, a temperature of less than 4°C must be maintained to prevent any bacteriological contamination.

Specific points:

The installation must be specially designed for each user.

Table 7 Change in the Fermenting Power of Different Quality Liquid Yeasts, Stored at Different Temperatures

Yeast quality	Length of storage, days	Loss of fermenting power lean dough, %		Loss of fermenting power sweet dough, %	
		4°C	12°C	4°C	12°C
Standard	7	0%	0%	0%	0%
	14	0%	0%	0%	0%
Rapid	7	0%	0%	0%	0%
	14	0%	0%	0%	0%
Ultra-rapid	7	0%	0%	0%	0%
	14	3%	4%	5%	10%
High activity A (Great Britain)[a]	7	0%	0%	—	—
	14	0%	4%	5%	10%
High activity B (Great Britain)[a]	7	0%	3%	5%	10%
	14	5%	30%	15%	50%

[a] Yeasts made using two different propagation methods. The stability of yeast B can only be achieved by positive temperatures of less than 4°C.

Profitability is guaranteed when the quantity of yeast used at the same site is sufficiently large.

Hygiene, cleaning, and disinfection conditions must be strictly observed.

The installation, made entirely of stainless steel, consists of two double-walled storage tanks with separate cooling systems, as well as feed systems for the tanks and mixers. The metering systems must be accurate and reliable. The whole unit is supplemented by a cleaning plant and equipment for discharging liquids: soda, antiseptics, rinsing water. The capacity of each tank must allow at least one week's production.

The liquid yeast is stirred in the tank and circulates continuously through the distribution system so that it remains homogeneous and there is no risk of infection. The temperature is strictly controlled and held at between 2 and 4°C. The system must be cleaned and disinfected once a week or whenever the installation is shut down, hence the need for two tanks. The receiving circuits must be cleaned each time there is a delivery. Procedures should be put in place and followed meticulously by operators.

2. Compressed Yeast

This is the most widely used form in industrialized countries, for economic and practical reasons. It comes in the form of compact blocks so that there is limited contact with the oxygen in the air. White in color and very friable in France, it can have a deeper color and a plastic consistency in other countries. The packaging of "waxed paper" or "sulfurized paper and Cellophane" restricts gaseous exchanges and controls the migration of moisture, to give a longer shelf life.

The stability of the fermenting power of yeast in its packaging depends on the storage temperature. One month is guaranteed for a "standard" quality French yeast stored at 10°C maximum (ideally 4°C). At 20°C, its activity is stable for 2 weeks but is reduced by 60% after 1 month; the DM loses 4 points, which corresponds to the consumption of storage sugars and to the product drying out; there is considerable contamination from mold. At 35°C, compressed yeast is only stable for 24 hr; after 3 days, the loss of activity is equivalent to that found after 1 month at 20°C and the product becomes brown, soft, and sticky.

The performance of "high activity" English yeast is guaranteed for 17 days if the storage temperature is strictly kept at a maximum of 2°C.

No special precautions are necessary when using compressed yeast: it can be easily crumbled into the mixer and there are no repercussions when it is dispersed in cold water. It should not be in contact with salt for too long, although this is not actually harmful in practice, contrary to received ideas.

Compressed yeast tolerates slow freezing very well (<1°C / min) and maintains its initial performance after a year's storage at −18°C (Table 8), provided that when it is used, it is thawed at positive low temperatures (°C) and is used within 24 hours. It is worth noting that there is an increase in the friability of yeast due to the evaporation of free water and a loss of dry matter as a result of storage sugar consumption.

3. Crumbled Yeast

This is in the form of relatively fine, free flowing particles, which means that it can be either weighed or measured automatically. It is packed in 25 kg, multilayer polyethylene-lined paper sacks, which are sealed to keep the product air-tight. Crumbled yeast is very sensitive to oxygen in the air because of its large surface of contact. Respiration which continues in a

Table 8 Change in the Properties of Compressed Yeast After Freezing

Properties	t 0	11 months at –18°C
Dry matter %	32.9	30.5
Nitrogen %/DM	7.2	—
Fermenting power %	100	97
Appearance	White, friable.	White, very friable, normal smell

very active manner after extrusion and during the bagging operation causes the yeast to heat up, making it very difficult to cool it down. However, the carbon dioxide released creates an inert atmosphere that stabilizes it.

The storage temperature must be under 10°C. Under these conditions, the crumbled yeast loses 5% of its initial fermentative power after a week and 10% after a month (losses are much higher in sweet dough).

Opened sacks should be used up within a day as the product heats up quickly (Table 9).

Crumbled yeast is often used by plant bakers as a suspension in water so that metering can be automated. The installation includes a storage tank with identical characteristics to those previously described for liquid yeast. Upstream, a smaller tank with a very vigorous stirring action is used to disperse the yeast in water.

4. Rehydratable Active Dry Yeast

Rehydratable active dry yeast is packed in air in 125 g or 500 g containers (metal or plastic), 10 or 25 kg sacks, or 25 kg plastic drums. It is packed in 5 to 11 g sachets for the consumer market. It is stored at ambient temperature. This rustic product is very stable, a fact which is appreciated in parts of the world where the climatic conditions are unfavorable (high temperature and humidity).

When reconstituted, the yeast undergoes a rehydration stage in about 5 times its weight of water, at a temperature between 35 and 42°C, the optimum being 38°C. Rehydration takes 15 min, during which time attempts should not be made to disperse

Table 9 Change in the Temperature of Crumbled Yeast, not Refrigerated

Conditions	Time	Temperature, °C
	t 0	8
	0H30	10
"Standard" quality yeast	1H00	14
Ambient temperature: 20°C	1H30	21
Quantity of yeast: 1.5 kg	2H00	26
Thickness of layer: 9 cm	—	—
	3H30	41
	4H30	49
	5H00	50
	5H30	47

the yeast mechanically or it will be damaged. After this resting time, it goes into suspension very easily and forms a cream.

Observing the rehydration temperature is vital because it ensures that the enzymatic mechanisms by which the cell membranes become semipermeable again are functioning properly. If the temperature is too low (<5°C), the membranes do not regain these properties quickly enough and the cells release their contents, resulting in their death. At 20°C, the loss of gassing power is about 30 to 40%.

In view of its dry matter content and fermentative capacity, its rate of substitution compared with "standard" quality French compressed yeast is about 40 to 50% (i.e. 1 kg of rehydratable dried yeast instead of 2 to 2.5 kg of compressed yeast).

Rehydratable dried yeast releases enough glutathione to change dough consistency and reduce its development time during mixing. Adjustments are therefore necessary; in particular, the quantity of oxidizing agent may need to be increased.

5. Instant Dry Yeast

Instant dry yeast vermicelli-like particles are *vacuum* packed, in 125 g or 500 g sachets, or in packs of 10 kg or more. There are also 7 to 12 g sachets packed with inert gas for home bread-making. The vacuum-packed sachets are hard: they ensure that the product is stable at ambient temperature. (Strict checks after packing eliminate sachets that no longer have this vacuum.) At temperatures of under 20°C, a loss of fermentative activity is apparent during the first month, and then stabilizes; after 1 year it is about 10 % ± 2. At 35 °C, one third of activity is lost in 1 year. Sachets that have been partially used should be kept in a cold place, carefully closed: the yeast's normal activity will be maintained for at least a week. Instant dry yeast owes its name to the fact that it does not need to be rehydrated before it is added to flour. It is as easy to use as compressed yeast. In most applications, the fineness and porosity of the particles mean that the yeast can be dispersed quickly and evenly in the dough. However, the use of very highspeed mixers (mixing times of less than 3 min) can pose some dispersion problems. Nevertheless, when using instant dry yeast, it should not come into direct contact with cold water, ice, or the refrigerated walls of mixers. It should just be mixed dry with the flour or sprinkled over the dough during mixing at first speed. In these conditions, the water heats up before it reaches the yeast cells whose membranes can regain their biological properties normally. However, it is less sensitive to cold than rehydratable dried yeast (−15% at 20°C/ 38°C).

The high fermentative power and dry matter content mean that it can be substituted at 33% for compressed yeasts with an equivalent composition. In other words: 1 kg of instant dry yeast can be used instead of 3 kg of compressed yeast. In view of the difference in dry matter content, it is essential to compensate for the difference in weight of water, (i.e., 2 kg in the example given).

As a small amount of glutathione may be released, it is advisable to reduce the mixing time slightly or increase the quantity of oxidizing agent in some applications. Because of the additional energy costs arising from drying and packaging technology, instant dry yeast is a product that cannot compete economically with compressed yeast when the latter is of good quality and the distribution networks are effective.

6. Free-Flowing Frozen Dry Yeast with Intermediate Moisture

In applications such as frozen dough, the results achieved with instant dry yeast are only comparable to those obtained with compressed yeast (CY) when the dough is stored for about 2 weeks. In fact, the yeast membranes, weakened by drying, cannot withstand being

frozen in the dough, then stored, and thawed again without damage. At lower dry matter contents ($\simeq$ 75 %), the yeast membranes remain intact. In its physical appearance as a powder, it has the characteristics of CY. It can be packed in air and frozen. It can be stored for long periods and exported to distant regions where there is no compressed yeast, and used in applications where instant dry yeast is unsuitable.

7. Dry Yeast with Reducing Power

a. Active In the form of small granules packed in air in plastic containers, this yeast is used by pizzerias. Its moderate, consistent fermenting power gives the tolerance during fermentation that is necessary for this type of application. The quantity of glutathione released means that pizzas can be easily molded, dough extensibility is increased, and shrinkage phenomena are reduced.

b. Deactivated This yeast has no fermenting capacity at all. In dough, the membranes that have been made completely permeable allow all the cell contents through including glutathione, which has a reducing effect on the gluten.

The quantities used vary from 0.3 to 1%, depending on the flour type and the effects required. The reducing effects work in the following way:

By improving the machinability of doughs that are too stiff or very strong.

By accelerating dough development during mixing, which requires a 15 to 20% reduction in mixing time. In the absence of lipoxygenase (from bean or soya flour), this property can be exploited to preserve the flavor of bread by limiting the oxidation of the flavor compounds.

Deactivated dried yeast is packed in polyethylene-lined paper sacks. Because the product is very hygroscopic, sacks should be closed carefully after use to prevent it becoming lumpy and to ensure it retains all its functions.

VI. CONCLUSIONS

Very little is known about yeast by the people who use it, and its true worth is certainly underestimated. Indeed, it is a product that has become banal in industrial countries, perhaps because the manufacturing process is well controlled and problems are therefore quite rare. Nevertheless, the yeast industry is a high-tech industry in the field of biotechnology where progress has been made due to intensive research and development work. As a result of genetic techniques, strains are being improved all the time. There have been advances in methods of culturing strains as we have a better understanding of biology and cell physiology. A good grasp of raw materials and manufacturing methods, intensive automation, and logistical controls are guarantees of product quality in a world increasingly concerned about food safety issues.

ACKNOWLEDGMENTS

To Eric Duquenne for his help with laying out the artwork. To Dorothy Ellerington, for her remarkable translation from French into good English of this unusual topic.

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Fermented Cereal-Based Functional Foods

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I. INTRODUCTION

Fermentation is one of the primary methods of adding value to foods, including improved palatability, safety, shelf life, and nutritional value. Fermentation can also be applied to designing and manufacturing of functional foods, which are defined as foods that are a normal part of the diet but that have components that exert a particular beneficial health effect on the consumer. Some of the major categories of functional foods, such as the probiotic foods, contain live microbes, and fermentation may produce or release potential health-promoting compounds in the substrate medium.

Among cereal-based foods, beer and bread are the main fermented products. In making beer and bread, alcoholic fermentation by yeast is the main type of fermentation; the yeast as live organisms are removed or killed in the final process. Lactic acid fermentation also occurs in many bread-making processes and in some beers, and the fermentation may result in beneficial modification of the cereal substrate.

In some other cereal processes, the lactic acid bacteria survive and live lactic acid bacteria are ingested with the cereal food. Currently it is not known if the various and undefined lactic acid bacteria present in traditional fermented cereal products have any probiotic effects, although this question could be raised on the basis of some reports available on fermented maize, sorghum, and millet (1).

The particular nutritional properties of oats as a potential functional food have created an interest in finding new food uses for oats, including fermented products. Work done in Finland and Sweden, the two major oat-producing and -exporting countries in Europe, has resulted in a new generation of oat products in the nondairy yogurt and beverage category. Defined probiotic strains have been used in the fermentation of oat-based cereal foods (2–5). Oat-based products also offer an alternative to soy-based fermented nondairy yogurts.

The present chapter describes some principles and examples of the use of cereal-based material for functional foods made with fermentation, including applications of probiotic lactic acid bacteria with cereal foods.

II. CEREAL AS A SUBSTRATE

Cereals are, in general, a good medium for microbial fermentations. They contain a high level of carbohydrates, which can be used as a source of carbon and energy by microbes in fermentation (Table 1). Most of the carbohydrate in cereals is present as starch and only available for microbes after amylolytic hydrolysis. The level of free sugars in fully matured sound grains is relatively low, but the 2–5% free sugars supports the initiation of the fermentation process. The content of free sugars in rye is higher than in other cereals, whereas in oats there are only 1–2% free sugars (10). Endogenous cereal enzymes, added malt, or selected enzymes can be used to break down the starch to simple fermentable sugars (i.e., maltose and glucose). There are also strains of amylolytic microbes, molds in particular, which produce amylases efficiently and can be used for the liquefaction and hydrolysis of starch, such as *Aspergillus* strains in the sake process (7). Lactic acid bacteria that are capable of breaking down and utilizing starch are also known (8,9).

Besides carbohydrates, cereals also contain relatively high levels of minerals, vitamins, sterols, and other growth factors, which support growth of microbes, including the fastidious lactic acid bacteria.

Cereals can offer several physiologically beneficial compounds for use in functional foods (12). In fact, some cereal products are declared functional as such. The beta-glucan soluble fiber present in oats has been of considerable interest in recent years. The U.S. Food and Drug Administration (FDA) has allowed the use of a health claim stating a connection between the soluble beta-glucan fiber from whole oats and reduced risk of coronary heart disease (13,14). A whole grain health claim stating a connection between whole grain intake and reduced risk of certain cancers has also been approved (15).

Soluble dietary fibers, such as the viscous oat beta-glucan, have also been shown to attenuate high glucose and insulin levels in blood serum (16). The hypoglycemic effects make oatmeal and oat bran even more interesting for the manufacturers of functional foods.

Some dietary fiber components, such as the soluble beta-glucan in oats and the soluble portion of arabinoxylans present in wheat and rye, have the ability to pass undegraded into the small intestine, thereby providing a substrate for the normal gut microflora, leading to the production of short chain fatty acids (SCFA). Of the SCFA formed, butyrate has in

Table 1 Generalized Compositional Data of Whole Dehulled Cereal Grains

Constituent	Content (%, dry matter basis)
Ash (minerals)	1.5–3
Protein	8–15
Lipids	2–6
Starch	45–77
Dietary fiber (as NSP + lignin)	9–12
Low-molecular-weight carbohydrates (total):	2–5
Fructose	0.1–0.4
Glucose	0.1–0.5
Sucrose	0.5–2
Raffinose	0.2–0.7

Source: Refs. 1, 6, 9–11.

particular been identified as being health promoting, mainly due to the stimulation of colonic crypt cells and the inhibition of colonic cancer cells (17). Arabinoxylan and beta-glucan may also have prebiotic effects supporting growth of beneficial bacteria in the colon (18).

III. TECHNOLOGIES AND EXAMPLES OF CEREAL-BASED FUNCTIONAL FOODS

Several technologies are available for manufacturing functional foods. The ILSI definition (19) refers to elimination or replacement of constituents with a negative contribution to nutritional value, or addition of positive components, or balancing the nutritional composition of the product. Table 2 lists some available techniques for making cereal based functional foods.

The nutritionally valuable components present in various cereal grains and their suitability for fermentation raise expectations about fermented cereal-based functional foods. However, only few such products have appeared on the market so far. Many possibilities for future development exist, some examples of which are listed below.

Folate content of bread is increased by fermentation of bread dough by yeast (20). Developing this technique might be of great importance because today a substantial part of the population does not meet the average recommended folate intake (21).

Phytate (myo-inositol hexaphosphate) constitutes 1–2% of cereal grains. It acts as the mineral and phosphor reserve of the seedling but is not hydrolyzed by the human digestive system. Although some recent studies suggest phytate to be a beneficial component, an improved bioavailability of minerals like zinc and magnesium would be desirable in strict vegetarian diets that are low in protein. Lactic acid fermentation in sourdough systems and indigenous fermented cereal foods will help in degradation of phytate (22–24).

Glycemia and insulinemia can be reduced by the lactic acid formed in wheat sourdough, according to a recent finding. The mechanism is not fully clarified but it is anticipated that the rate of starch digestion is reduced because of increased gluten–starch interactions (25).

Exopolysaccharides formed by lactic acid bacteria have been of interest for their technological potential as thickening agents, or as a possible source of ropiness, and for their potential as dietary fiber (26).

Bioactive peptides with antihypertensive properties are formed in a number of foods during wet-processing such as fermentation. Recent experimental work suggests that peptides capable of inhibiting the angiotensin I converting enzyme are present also in wheat (27).

Table 2 Possible Techniques for Making Cereal-Based Functional Foods

Rely on cereal compounds as such (e.g., dietary fiber component, cf. FDA)
Enrich with an incorporated external ingredient (e.g., vitamin, mineral)
Add a probiotic (e.g., a strain of *Lactobacillus* or *Bifidobacterium*)
Modify a functional compound enzymically (e.g., release of bioactive, minerals, prebiotic compounds, phytoestrogen precursors)
Synthesize a functional compound by fermentation (e.g., a vitamin)
Combine two or more of the above techniques

Table 3 Examples of Cereal-Based and Cereal-Related Foods Containing Live Probiotic Bacteria

Product	Preparation method and type of product	Bacteria used and levels	Purported physiological benefit(s)	Reference
Togwa	Sorghum or maize cooked in water, cooled, addition of starter, fermentation. Traditional weaning food, beverage after dilution	Dominated by *Lactobacillus plantarum*, also *L. brevis*, *L. fermentum*, *Pediococcus pentosaceus*, 10^9 cfu/g. Strains from local starters.	Reduces enteropathogen occurence in rectal swabs of children, improves intestinal mucosa barrier function in children with acute diarrhea.	29, 30
Fermented oatmeal soup for enteral feeding	Oatmeal cooked with malted barley flour, cooled, addition of starter, fermentation. Designed for enteral feeding	Probiotic *Lactobacillus* strains, development made with *L. plantarum* 299v, *L. reuteri*, and other strains. Strains protected.	Maintains intestinal function and structure after surgical operations, diarrhea, etc.; strains able to colonize human intestinal mucosa; reduction of sulfite-reducing clostridia, enterobacteria, and other undesirable bacteria.	2, 4, 31, 32
Fermented fruit drink with oatmeal	Oatmeal and water-heated, cooled, fermented, cooled and mixed with flavored fruit drink. Snack-type nondairy beverage (Proviva®)	*Lactobacillus plantarum* 299v, 5×10^7 cfu/g. Strain protected.	Strain able to colonize in the intestinal mucosa and has been associated with a reduction in harmful bacteria. Lead to increase in SCFAs, which are an energy source for mucosal cells.	5, 34
Oat bran vellie	Oat bran and water cooked, cooled, fermented, cooled and flavored with fruit, berries. Yogurt-type nondairy snack (Yosa®)	*Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* Bb-12, and other strains, 5×10^7 cfu/g. Only cereal-based product with bifidobacteria. Strains commercially available.	Potential synbiotic effects with probiotic and prebiotic components combined. Up to 0.5% viscous beta-glucan soluble fiber, which may contribute to cholesterol lowering and blood glucose attenuation.	33, 35

The anecdotal and documented benefits of some traditional fermented foods, such as Tanzanian *togwa*, may well place them in the category of probiotic food (28). Togwa is a lactic acid fermented sorghum or maize gruel that is used as a weaning food or can be diluted and used as a beverage (29). Unlike most fermented cereal products, togwa is not heat treated after fermentation, and therefore it contains live lactic acid bacteria, mainly *L. plantarum*. Fermenting *togwa* inhibits the growth of some enterotoxin-producing bacteria (28). Further, a significant reduction in the enteropathogen occurrence in rectal swabs of children younger than 5 years was achieved when the children were fed togwa (30).

The benefits of defined probiotic bacteria strains, such as *Lactobacillus acidophilus* and *Bifidobacterium lactis*, are well documented. However, these strains have been most commonly utilized in dairy-based foods. Only the past decade has shown that probiotic strains can also be incorporated in cereal-based media.

Table 3 describes examples of cereal-based foods and cereal-related foods containing live probiotic bacteria. A crucial determinant of the usefulness and effectiveness of probiotics in fermented foods is their ability to grow in the medium during fermentation and to survive in the product during storage. Oat-based media have been shown to support the growth of probiotics during fermentation (2,3,5,26,31,33). It has also been shown that the applicable strains were capable of maintaining high (10^8 cfu/g) cell counts during cold storage for several weeks. These cell counts are also typical to vellie, a fermented oat bran-based porridge. Refrigerated storage for several weeks does not affect drastically the viability of the key strains in these products.

IV. CONCLUSIONS

Fermented cereal-based foods have a long tradition, although some of the processes may almost be forgotten. However, the new information on the benefits of probiotic strains has also spurred an interest in the research and development of cereal-based probiotic carrier foods. Fermentation also provides possibilities for physicochemical (solubility) and enzymatic modifications of the cereal substrate, and therefore further development of the processes and products may also lead to enhancement of the health-promoting functional properties of the food.

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Sourdough Bread

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I. INTRODUCTION

Sourdough is used as an essential ingredient in the production of wheat and rye bread and mixtures thereof. Sourdough has been used for leavening of bread dough for several hundreds of years, and sourdough bread was made in Egypt as early as 3000 B.C. (1). The sourdough was a piece of dough from the previous baking that was kept until the next baking, when it was mixed with flour, salt, and water to make the bread dough. The intervals between baking could be from 1 day, in bakeries, to 1 month, in home baking. If the time between baking was long, salt could be added to the surface of the sourdough to avoid wrong fermentation. While this piece of dough was saved, lactic acid fermentation took place due to multiplication and metabolic activity of lactic acid bacteria (LAB) originally present in the flour. During this fermentation, selection and multiplication of yeasts from the flour also occurred. The natural content of LAB and yeasts from the sourdough was responsible for the leavening capacity of the bread dough primarily due to their production of carbon dioxide. Yeast from beer or wine production could also be added to the dough to increase the leavening capacity until production of commercial baker's yeast began during the nineteenth century (2). The sourdough still holds a place of honor in many households throughout the world, and small portions are passed on to the daughters at marriage (2).

A. Wheat Sourdough

Sourdough is used as an important ingredient in the production of wheat bread (3,4) as well as crackers and the Italian sweet baked products as pandoro, colomba, and panettone (5,6). The tradition of making wheat bread with the addition of sourdough is widely used in the Mediterranean area, as in Italy (5,7), Greece (8,9), Spain (10), Egypt (11), and Morocco (12). The tradition is also known in The Netherlands (13), Iran (14), and the San Francisco Bay area in the United States (15,16).

The cereal intake in the traditional diet of Greece is mostly in the form of sourdough bread rather than pasta (8). In Italy, sourdough is used in more than 30% of bakery products, which include more than 200 different types of sourdough bread. In some regions of southern Italy, most of the bread, including sourdough bread, is made from durum wheat instead of common bread wheat (7). In Morocco, commercial bakeries supply only part of the population with bread; most people eat home-made bread made with traditional

sourdough, which has been carefully kept in every family. Addition of baker's yeast is used mainly in towns and villages where refrigeration can be employed (12).

B. Rye Sourdough

Sourdough is essential in rye bread making, and the tradition of rye sourdough fermentation corresponds to the rye-growing areas in the north, central, and eastern European countries, including the Baltic States, where rye bread constitutes a considerable amount of the bread consumption. Rye sourdoughs have been characterized from Finland (17), Sweden (18), Denmark (19,20), Germany (21–23), Austria (24), Poland (25), Czechoslovakia (26), Russia (27), and Portugal (28).

Bread made from mixed wheat and rye is very common in many European countries, and sourdough should be used to enhance the sensory properties of the bread and prolong the microbial shelf life if more than 20% of the flour is from rye (29). One of the most famous rye sourdough breads still produced today is Pumpernickel, named after the Swiss baker Pumper Nickel. The bread originated in 1443, when there was a significant scarcity of wheat in Europe (2).

The tradition of production of rye bread without the addition of baker's yeast has continued even in large-scale bakeries until today, and the leavening capacity of the sourdough is still very important in rye bread production. In the 1960s and 1970s, the time between baking and consumption of bread increased due to changes in society; and in some bakeries, preservative compounds such as vinegar, propionic acid, or sorbic acid were added to the dough for the prevention of molds. However, the natural content of yeasts from the sourdough is also inhibited by those preservatives, resulting in decreased leavening capacity, and it was necessary to add baker's yeast to increase the bread volume. The use of propionic acid as a preservative in bread is prohibited in many countries today. Stringent hygiene in bakeries makes it possible to produce bread with long shelf life without added preservatives, if sourdough is added.

C. Why Is Sourdough Used?

The advantages of using sourdough for bread production include the possibility of leavening bread dough with little or no baker's yeast added, improved dough properties, and the achievement of a better and more aromatic bread flavor and texture compared to bread leavened only by baker's yeast (Table 1). Sourdough flavour is developed by a long fermentation process that requires 12–24 hr, whereas fermentation by baker's yeast has to be finished within 1–2 hr. The addition of sourdough can also extend the shelf life of bread by several days by increasing the mold-free period of bread and retarding the development of rope. The nutritional value of sourdough bread made from high-extraction flour is enhanced compared to bread made without sourdough because of a higher content of free minerals, which are separated from phytic acid during the long fermentation processes.

Interest in using sourdough in bread production has increased considerably in many European countries during recent decades (4,30,31). Today, more consumers prefer healthy bread with aromatic taste, good texture, and long shelf life without the addition of artificial preservatives. The demand for organic food is also on the rise, and a larger part of the bread made from organically grown cereals is made with sourdough due to its higher quality and better image. More consumers are also interested in food with a history, and sourdough bread is related to traditional and original food.

Table 1 The Advantages in Using Sourdough in Bread Making

Leavening of dough
Improved dough properties
Inhibition of α-amylase
Increased flavor and taste of bread
Improved nutritional value of sourdough bread
Higher bioavailability of minerals
Lower glycemic index
Extended shelf life of sourdough bread
Longer mold-free period
Prevention of rope in bread
Anti-staling

II. CHARACTERIZATION OF SOURDOUGH

High-quality sourdough bread is dependent on a consistent and microbially stable sourdough. Good fermentation capacity of the sourdough is influenced by the microbial flora (lactic acid bacteria and yeasts) in the sourdough, flour type (wheat/rye, flour extraction rate, activity of enzymes), flour/water ratio (dough yield), and the process parameters. The process parameters such as temperature, initial pH, quantity of added sourdough starter, time of fermentation, and type of production system (batch/continuous) have to be strictly controlled.

A. Definition of Sourdough

Sourdough is a mixture of flour and water in which LAB have caused a lactic acid fermentation to occur. It is in general accepted that the LAB should still be able to produce acids when flour and water are added (metabolically active). The sourdough also has a natural content of sourdough yeasts, which are important for the leavening capacity of the dough. However, no official definition of sourdough exists, but it should include all different types of sourdough products with "living" LAB and excludes artificial sourdough products. According to Lönner (32), a sourdough should contain more than 5×10^8 metabolically active LAB/g, and have a pH value below 4.5.

B. Types of Sourdoughs

Sourdoughs can be started as follows:

During spontaneous fermentation
By adding a piece of mature sourdough (mother sponge)
By adding a defined starter culture

Most sourdoughs used in both wheat and rye bread baking are still initiated by adding a piece of mature or ripe sourdough, also called mother sponge, but there is a tendency to use defined starter cultures with specific fermentation patterns or production of antimicrobial substances. This tendency increases as these cultures become commercially available.

1. Spontaneous Fermentation

When dough made from flour and water is left for 1 to 2 days at ambient temperature, a spontaneous fermentation will take place due to the naturally occurring microorganisms in the flour. The dough will become acidified due to lactic acid fermentation. During the fermentation there is a successive favoring of the gram-positive LAB from the flour at the expense of the gram-negative bacteria that dominate the microflora of the flour (21,32). The microflora of some spontaneously fermented rye sourdoughs were dominated by homofermentative *Lactobacillus* spp. and *Pediococcus* spp. (32,33). The level of LAB in sourdoughs was up to 3×10^9 colony forming units (CFU)/g and the number of yeasts about 10^6 to 10^7 CFU/g. However, spontaneous sourdoughs do not always succeed and may result in products with off-flavor.

2. Mature Sourdough

Sourdoughs used by artisan bakers and in bakeries have traditionally been based on spontaneous fermentation, during which the sourdough has been kept metabolically active and probably microbially stable for decades by the daily addition of flour and water, the so-called freshening of the dough based on back-slopping (Fig. 1). The fermented sourdough is used for bread production, but part of it is used as starter by initiating a new sourdough. The terminology for sourdough and starter in different countries is listed in Table 2.

In commercial rye bread baking, bakeries can use their own adapted sourdough or, if they have quality problems due to unstable process control, they can add a commercial sourdough as a starter. Most bakeries in Germany and Denmark regularly add commercial sourdoughs composed of a well-adapted microflora derived from natural sourdough fermentation. Examples of commercial sourdoughs are the Sanfrancisco sour for wheat bread production (34) and the Böcker-Reinzucht-Sauer® for rye bread production. Some prod-

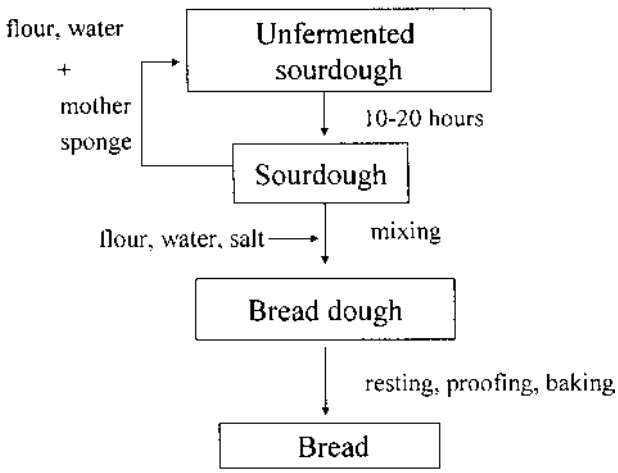

Figure 1 A schedule for production of sourdough and sourdough bread.

Table 2 Terminology for Sourdough in Different Countries

	English	German	French	Spanish	Italian
Sourdough for bread production	Sourdough, leaven	Sauerteig	Levain natural	Masa madre (masa agria)	Lievito naturale (impasto acido)
Sourdough used as starter for a new sourdough	Mother sponge, starter	Anstellgut Reinzuchtsauer®	Le chef	Pie	Madre, capolievieto

Source: Ref. 5.

ucts sold as sourdough have no living microorganisms, and these products will not contribute to a natural acidification and development of flavor compounds in the dough.

3. Starter Cultures of Pure Strains of LAB

Starter cultures for sourdough fermentation are pure cultures of dried or freeze-dried LAB, or a mixture of LAB and sourdough yeast. They should be mixed with flour and water, and kept for several hours for multiplication and fermentation of the microflora. This fermented dough can then be used as a sourdough. The microorganisms have been selected due to their ability to acidify dough in a short time and result in acceptable bread flavor when used in bread baking. Cultures containing *Lactobacillus sanfranciscensis*, *L. plantarum*, *L. brevis*, and *L. fructivorans* or *L. brevis*, *L. pontis*, and *S. cervisiae* are available (35). Use of defined starter cultures with specific properties gives rise to new interesting opportunities for controlling and regulating sourdough fermentation. The term "starter culture" is sometimes used in the literature for a mature sourdough that has to be mixed with flour and water to ferment, or for commercial sourdoughs.

C. Sourdough Parameters

A sourdough can be characterized by the chemical parameters—pH, content of total titratable acids (TTA), content of lactic and acetic acid—and the microbial parameters, such as number and species of LAB and yeast. The microbial parameters are described in the following section.

The final pH of a mature sourdough is 3.5–3.8 in most rye and wheat sourdoughs (3,36,37). Sourdough pH values show less variation and differences than TTA values. The TTA values in sourdoughs are dependent on the fermentation temperature, extraction rate of the flour, and the water content. In wheat sourdoughs, TTA has been found to vary between 8 and 11 in sourdoughs made from low extraction flour and 16 to 22 in wholemeal sourdoughs (3,38). Rye sourdoughs are often made from flours with higher extraction rate than wheat flour, and TTA values vary between 15 and 26 (21,36).

The content of lactic and acetic acid in sourdoughs is very important for the taste and flavor of sourdough bread (31,39). The fermentation quotient (FQ), the molar ratio between lactic and acetic acid, is used as a measure in German studies of sourdoughs for the balance in production of those acids (21). The FQ should be around 4 in sourdough to result in a bread with a well-balanced taste. A low content of acetic acid results in a high FQ with a too little flavor, whereas a low FQ results in too strong an acid flavor (21). However, acetic acid has a more efficient antimicrobial effect against mold- and rope-producing bacteria than lactic acid (40).

Table 3 Lactic Acid Bacteria and Yeasts Isolated from Rye Sourdoughs

Country	Lactic acid bacteria		Yeasts	Authors	Year	Reference
	Homoferm.	Heteroferm.				
Russia	*Streptobacterium plantarum* (*L. plantarum*)	*L brevis* *L. fermenti*	Nonidentified species	Sseliber	1939	Cit. from (43)
Czechoslovakia	*Str. plantarum*	Nonidentified species	*S. cerevisiae*	Pokorny	1955	Cit. from (43)
Germany	*L. delbrueckii* *L. plantarum* *L. leichmanii*	*L. brevis* *L. fermenti*		Spicher	1959	(43)
Germany	*L. alimentarius* *L. plantarum* *L. acidophilus* *L. casei* *L. farciminis*	*L. brevis* *L. brevis* subsp. *lindneri* *L. plantarum* *L. fermentum* *L. fructivorans*		Spicher, Schröder	1978	(119)
Germany			*C. krusei* *S. cerevisiae* *Pichia saitoi* *T. holmii*	Spicher, Schroeder, Schoellhammer	1979	(120)
Austria	*L. alimentarius* *L. casei* *L. rhamnosus*	*L. brevis* subsp. *lindneri* *L. büchnerii* *L. fermentum* *L. fructivorans*	*S. cerevisiae* *C. krusei*	Formatti, Mar	1982	(24)
Russia	*L. plantarum* (firm) *L. plantarum* (liquid) *L. leichmanii* *L. casei* var. *casei* *L. delbruckii* (48–52°C)	*L. brevis* *L. fermenti* *L. brevis* *L. büchnerii*	*S. minor*/*S. exiguus* *S. minor* *S. exiguus*	Kazanskaya, Afanasyeva, Patt	1983	(27)
Finland	*L. acidophilus* *L. plantarum*	*L. büchnerii* *L. cellobiosus* *L. viridescens*		Salovaara, Katunpäa	1984	(17)
Finland			*Torulopsis holmii* *S. cerevisiae* *T. unisporus* *T. stellata* *Endomycopsis fibuliger* *Hansenula anomala*	Salovaara, Savolainen	1984	(57)
Sweden	*L. acidophilus* *L. plantarum* *L. farciminis* *L. casei rhamnosus* *L. delbruckii* (53°C)	*L. brevis* *L. brevis* subsp. *lindneri* *L. fermentum* *L. viridescens*		Spicher; Lönner	1985	(18)
Poland			*S. cerevisiae* *S. exiguus* *T. candida* *C. krusei*	Wlodarczyk	1986	(25)

Table 3 Continued

Country	Lactic acid bacteria: Homoferm.	Lactic acid bacteria: Heteroferm.	Yeasts	Authors	Year	Reference
Germany		*L. brevis* *L. sanfrancisco*		Böcker, Hammes	1990	(121)
		L. curvatus *L. sanfrancisco*		Okada, Ishikawa, Yoshida, Uchimura, Ohara, Kozaki	1992	(122)
Germany		Nonidentified species closely related to *L. fermentum* and *L. reuteri*	*S. cerevisiae*	Strohmar, Diekman	1992	(42,123)
Germany		*L. brevis* *L. sanfrancisco* *L. fermentum* *L. fructivorans* *L. pontis* sp. *nov.*		Vogel, Bocker, Stolz, Ehrmann, Fanta, Ludwig, Pot, Kersters, Schleifer, Hammes	1994	(49)
Portugal (rye and maize)			*S. cerevisiae* *Torulaspora delbrueckii* *Issatchenkia orientalis* *Pichia anomala* *P. membranaefaciens*	Almaida, Pais	1996	(58)
Germany		*L. panis* sp. *nov.*		Wiese et al.	1996	(50)
Finland			*C. milleri* *S. cerevisiae* *S. exiguus*	Mäntynen et al.	1999	(55)
Germany		*L. frumenti* sp. *nov.*		Müller, Ehrmann, Vogel	2000	(52)
Denmark	*L. acidophilus* (liquid) *L. amylovorus*	*L. panis*	*S. cerevisiae*	Rosenquist, Hansen	2000	(20)
Germany	*L. mindensis* sp.*nov.*			Ehrmann et al.	2003	(53)

L., *Lactobacillus*; *Str.*, *Streptobacterium*; *S. Saccharomyces*, *C.*, *Candida*; *T. Torulopsis*;

III. MICROBIOLOGY OF SOURDOUGH

The microflora of the sourdoughs includes adapted LAB and yeasts that have optimal conditions for growth and fermentation similar to the conditions for the sourdough (temperature, water content, pH), and which probably produce antimicrobial compounds (41). The microflora in bakery sourdoughs remains remarkably stable despite the use of nonaseptic fermentation conditions (20,23,34,42). The LAB and yeasts isolated and identified from rye and wheat sourdoughs are listed in Tables 3 and 4, respectively.

Early systematic studies of the microflora responsible for sourdough fermentation were made on sourdoughs from Germany by Hollinger in 1902, from Russia by Sseliber in 1939 [cited in (43)], and from Denmark by Knudsen in 1924 (19). Spicher and coworkers have carried out many thorough investigations concerning the identification of the microflora from different types of sourdoughs, both adapted sourdoughs from bakeries and in commercial starter cultures for sourdough (4,21). Recent investigations on sourdough

Table 4 Lactic Acid Bacteria and Yeasts Isolated from Wheat Sourdoughs

Product	Lactic acid bacteria		Yeasts	Authors	Year	Reference
	Homoferm.	Heteroferm.				
San Francisco Bread		*L. sanfrancisco*	*S. exiguus*	Kline, Sugihara, McCready	1971	(15)
			S. inusitas	Sugihara et al.	1971	(34)
Balady bread				Abd-el-Malek, El-Leithy, Awad	1974	(11)
Sangak bread	*L. plantarum*	*L. brevis*	*T. collucolasa*	Azar, Ter Sarkissian, Ghavifek, Ferguson, Ghassemi	1977	(14)
		Lc. mesenteroides	*T. candida*			
Wheat bread	*L. plantarum*	*L. brevis*		Spicher, Lönner	1985	(18)
Wheat bread	*L. plantarum* *L. farciminis* *L. casei*	*L. brevis*		Spicher	1987	(124)
Wheat bread		*L. brevis* var. *lindneri* *L. sanfrancisco*	*S. exiguus*		1987	(13)
		L. brevis var. *lindneri*	*S. exiguus*	Nout, Creemer-Molenaar		
Swiss panettone/cake		*L. brevis* var. *lindneri*		Spicher	1987	(124)
Swiss bread		*L. brevis* var. *lindneri*		Spicher	1987	(124)
Panettone, brioches, wheat bread, crackers	*L. plantarum* *Pediococcus*	*L. sanfrancisco* *L. fermentum* *Lc. mesenteroides*	*S. cerevisiae* *C. stellata* *C. milleri*	Galli et al.	1988	(125)
Wheat bread	*L. plantarum* *Lc. mesenteroides*	*L. brevis* *L. cellobiosus*	*S.cerevisiae* *C. boidinii* *C. guilliermondii* *Rhodutorula glutinis* *Pichia polymorpha* *Tricocporon margaritiferum*	Barber, Baguena	1988	(10)
Wheat bread	*L. plantarum* *L. delbrueckii* *Lactococcus casei*	*L. brevis*	*C. milleri* *S. cerevisiae*	Boraam et al.	1993	(126)

Pannettone, bread	*L. plantarum*	*L. brevis* var. *lindneri*	*S. cerevisiae*	Gobbetti, Corsetti, Rossi, Rosa, Vincenzi-S-de.	1994	(127)
	L. farciminis		*S. exiguus* *C. krusei*			
Bread			*S. cerevisiae* *S. exiguus* *C. krusei* *Pichia norvegensis* *Hansenula anomala*	Rossi	1996	(59)
Maize bread	*L. delbrueckii* *L. curvatus* *L. plantarum* *L. lactis* spp. *Lactis*	*L. brevis*	*S. cerevisiae*	Rocha & Malcata	1999	(28)
Wheat bread	*L. paralimentarius* sp. *nov.*			Cai, Okada, Mori, Benno, Nakase	1999	(28,51)
Cakes: panettone, colomba, brioche		*L. sanfranciscensis*	*C. holmii*	Foschino, Terraneo, Mora, Galli	1999	(28,60)
		L. brevis				
	L. delbrueckii	*L. sanfranciscensis*	*S. cerevisiae*			
	L. alimentarius	*L. sanfranciscensis*	*S. cerevisiae*	Corsetti, Lavermicocca, Morea, Baruzzi, Tosti, Gobbetti	2001	(7)
Bread—durum wheat and bread wheat	*L. plantarum*	*L. brevis* *Lc. citreum* *L. fermentum*				
Wheat bread	*L. paralimentarius*	*L. sanfranciscensis*		De Vuyst, Schrijvers, Paramithiotis, Hoste, Vancanneyt, Swings, Kalantzopoulos, Tsakalidou, Messens	2003	(9)
	Weissella cibaria	*L. brevis*				

L., *Lactobacillus*; *S. Saccharomyces*, *C.*, *Candida*; *T. Torulopsis*

fermentation have mainly dealt with interactions between sourdough microorganisms, identification of new species, inhibitory substances of sourdough LAB, and induced specific enzymatic activities.

LAB are mainly responsible for the acidification of the sourdough, whereas the sourdough yeasts are very important for the production of flavor compounds and for a well-balanced bread flavor in combination with the acids (31). The levels of LAB in sourdoughs are 10^8–10^9 CFU/g and yeasts are 10^6–10^7 CFU/g, respectively (4). The LAB: yeast ratio in sourdoughs is generally 100:1.

A. Lactic Acid Bacteria

1. Identification

LAB are a group of gram-positive bacteria, which are catalase-negative, nonmotile non-sporeforming rods or cocci that produce lactic acid as the major end product during the fermentation of carbohydrates. They are strictly fermentative, aerotolerant or micro-aerofile, acidophilic, salt-tolerant and have complex nutritional requirements for carbohydrates, amino acids, peptides, fatty acids, salts, nucleic acids derivatives, and vitamins (44,45).

The LAB have traditionally been classified taxonomically into different genera based on colony and cell morphology, sugar fermentation, growth at different temperatures, configuration of lactic acid produced, ability to grow at high salt concentration, acid tolerance, or cell wall analyses (46). Genera of LAB identified from sourdoughs are *Lactobacillus*, *Leuconostoc, Pediococcus*, *Lactococcus*, and *Weisella*, and the majority of the sourdough LAB belongs to the genus *Lactobacillus*. The taxonomy of LAB is still under revision. *Lactobacillus* have been divided into three groups according to their carbohydrate fermentation patterns (46):

Obligately homofermentative LAB: Hexoses are almost completely fermented to lactic acid (>85%) by the Embden-Meyerhof-Parnas (EMP) pathway. Fructose is also fermented, but neither gluconate nor pentoses are fermented.

Facultatively heterofermentative LAB: Hexoses are almost completely fermented to lactic acid by the EMP pathway. Pentoses are fermented to lactic acid and acetic acid by an inducible phosphoketolase.

Obligately heterofermentative LAB: Hexoses are fermented to lactic acid, acetic acid (ethanol) and CO_2. Pentoses are fermented to lactic and acetic acid. In general, both pathways involve phosphoketolase.

Lactobacillus isolated from sourdoughs are divided into the three groups shown in Table 5. When the LAB are only divided as homofermentative or heterofermentative LAB, the facultative heterofermentative LAB are grouped as homofermentative due to the fermentation pathway of glucose (Tables 3 and 4).

In the presence of oxygen or other oxidants, increased amounts of acetate may be produced at the expense of lactate or ethanol (45). Various compounds such as citrate, malate, tartrate, quinolate, and nitrate may also be metabolized and used as energy sources or electron acceptors (44,45).

The traditional techniques used for classification of LAB are not reliable for many LAB, and they have often led to misidentification (47). Some organisms grow poorly on laboratory media and may escape isolation and can therefore not be identified by standard procedures. For rapid classification, a set of molecular probes was developed. These include hybridization- and PCR-based techniques as well as recognizing specific sequences in the ribosomal genes (47). Nevertheless, the use of probes at the species level is restricted, as their

Table 5 Groups of Lactobacillus Isolated from Sourdoughs

Characteristics	Obligately homofermentative	Facultatively heterofermentative	Obligately heterofermentative
Growth at 15°C	−	+	+/−
Growth at 45°C	+	−	+/−
Pentose fermentation	−	+	+
CO_2 from glucose	−	−	+
CO_2 from gluconate	−	+[a]	+[a]
FDP[b] aldolase present	+	+	−
Phosphoketolase present	−	+[c]	+
Lactobacillus	*L. acidophilus*	*L. alimentarius*	*L. brevis*
	L. amylovorus	*L. casei*	*L. buchneri*
	L. delbrueckii spp. *bulgaricus*	*L. curvatus*	*L. fermentum*
	L. delbrueckii spp. *delbrueckii*	*L. paralimentarius*	*L. fructivorans*
	L. farciminis	*L. plantarum*	*L. frumenti*
	L. helviticus	*L. rhamnosus*	*L. hilgardii*
	L. leichmanni		*L. panis*
	L. mindensis		*L. pontis*
			L. reuteri
			L. sanfranciscensis[d]
			L. viridescens

[a] When fermented.
[b] FDP fructose-1,6-diphosphate.
[c] Inducible by pentoses
[d] Former names of some of the bacteria: *L. brevis* spp. *lindneri* and *L. sanfrancisco*.
Source: Ref. 46.

specificity may be lost during discovery of new species sharing the same part of an RNA sequence (48). Alternatively, the taxonomic method Random Amplified Polymorphic DNA (RAPD) allows elucidation of strain biodiversity below the species level, and the resulting electrophoretic patterns can be clustered and compared to a database (48). The consequent application of 16S rRNA sequence analysis and DNA-DNA hybridization experiments has led to identification of many new species. *L. pontis* (49) and *L. panis* (50) were isolated from rye sourdoughs, and *L. paralimentarius* (51) was isolated from wheat sourdough. Recently described species isolated from sourdoughs are *L. frumenti* (52) and *L. mindensis* sp. *nov* (53).

2. Occurrence

Heterofermentative LAB play a major role in sourdough fermentation compared to other fermented food systems. *L. sanfranciscensis* (former names *L. brevis* var. *lindneri* and *L. sanfrancisco*) is by far the most dominant LAB in both rye and wheat sourdoughs (Tables 3 and 4). *L. brevis* and *L. plantarum* also occur frequently in both types of sourdoughs. Some strains initially classified as *L. brevis* were renamed as *L. pontis* (49). Several other lactobacilli have been identified from rye sourdoughs, for example, the homofermentative *L. acidophilus*, *L. alimentarius*, *L. amylovorus*, *L. casei*, *L. delbrueckii*, *L. farciminis*, *L. leichmanii*, *L. rhamnosus*, and recently *L. mindensis*, and the heterofermentative *L. büchnerii*, *L. cellobiosus*, *L. curvatus*, *L. fructivorans*, *L. fermentum*, and *L. viridescens*, including the

newly identified species *L. panis*, *L. frumenti,* and *L. pontis*. Strains of *L. pontis* utilize only a very limited number of carbohydrates, and they are found in close association with *L. sanfranciscensis*, from which they are difficult to separate physically.

Fewer different lactobacilli have been identified from wheat sourdoughs, such as *L. alimentarius*, *L. casei*, *L. cellobiosus*, *L. curvatus L. delbrueckii*, *L. farciminis*, *L. fermentum*, *L. lactis,* and the recently identified *L. paralimentarius* (Table 4). However, the homofermentative *Pediococcus* and *Weissella* and the heterofermentative *Leuconostoc* have also been isolated from wheat sourdoughs.

The variation in the composition of the microflora depends on the fermentation conditions, such as flour type, extraction rate, water content, fermentation temperature, fermentation time, and how the sourdough is refreshed. Most sourdoughs are fermented at about 30 °C, but *L. delbrueckii* has been isolated from rye sourdoughs with a fermentation temperature above 50°C (18,27). Hammes and Gänzle (35) found that the most predominant LAB in firm sourdoughs with fermentation temperature between 23 and 30°C are *L. sanfranciscensis* and *L. pontis*. However, *L. fructivorans*, *L. fermentum*, and *L. brevis* were also identified from this type of sourdough.

Some industrial sourdoughs are characterized by high water content to fluid conditions (suitable for pumping), elevated fermentation temperature (>30°C), and shorter fermentation time (15–20 hr). Fluid sourdoughs can be produced in large volumes, often by continuous fermentation systems, and they can be cooled for storing in silos up to 1 week. The microflora in this type of sourdough is dominated by *L. panis* (50), *L. reuteri, L. sonfranciscensis*, and *L. pontis* (35). The development of sourdough yeast is poor in fluid sourdoughs, and consequently it is necessary to add baker's yeast to the bread dough.

Sourdoughs kept at ambient temperature will continue acidification. The LAB are sensitive to low pH in longer time and the LAB will thus die off. Therefore, dried sourdough preparations are preferred for commercial sourdough samples. However, LAB are rather sensitive to preservation by drying, and LAB present in commercial sourdoughs must survive drying. *L. plantarum*, *L. brevis*, *Pediococcus pentosaceus* have been identified from dried commercial sourdough preparations, and dried starter cultures containing strains of *L. sanfranciscensis* have only recently become commercially available (35).

B. Yeast in Sourdoughs

Several species of yeasts have been isolated from bakery and commercial sourdoughs. However, the taxonomy of yeasts has been gradually changed since the 1970s, and various synonyms have been used (Table 6) The traditional systematization and identification of yeasts have been based on biochemical tests as well as morphological and physiological criteria (54), but imperfect fungi cannot be studied using traditional genetics. New molecular characteristics have defined and changed the taxonomy of yeasts (55). The physiological features of industrial yeasts have been shown to alter when changes occur in growth conditions, and species of *Saccharomyces cerevisiae*, *S. exiguous*, and *Torulopsis delbrueckii* have been found to intermix genetically with each other (55).

The most frequently isolated yeast species from rye and wheat sourdoughs are *S. cerevisiae* (Tables 3 and 4). Other yeast species often isolated from sourdoughs are *S. exiguus, Candida milleri* (*C. holmii*), and *C. krusei* (*Issatchenkia orientalis*). The yeast species *Pichia saitoi*, *P. norvegensis*, and *Hansenula anomala* and some *Saccharomyces* spp. have occasionally been isolated from sourdoughs.

Candida spp. are members of *Deuteromycetes* (fungi imperfect) because they have lost their ability to undergo sexual development. *C. milleri* is a nonsporulating form of

Table 6 Yeasts Isolated from Sourdoughs and Their Synonyms

Perfect fungi	Imperfect fungi	Synonyms
Saccharomyces cerevisiae		
S. exiguus	*Candida holmii*	*Torulopsis holmii*
		Torula holmii
		S. rosei
	C. milleri	*Torulopsis holmii*
S. delbrueckii		*Torulaspora delbrueckii*
S. uvarum		*S. inusitatus*
Issatchenkia orientalis	*C. krusei*	*S. krusei*
		Endomyces krusei
Pichia anomala	*C. peliculosa*	*Hansenula anomala*
P. membrafaciens	*C. valida*	
P. norvegensis		
P. polymorpha		
P. satoi		
Endomycopsis fibuligera		*S. fibuliger*

C., *Candida P.*, *Pichia*; *S. Saccharomyces*
Source: Refs. 128 and 129.

S. exiguous and was first described by Yarrow in 1978 (56). *C. milleri* is physiologically similar to *C. holmii* but different according to DNA identification. Some strains identified as *Torulopsis holmii* in the literature before 1978 have subsequently been assigned to *C. milleri* (55).

T. holmii and *S. cerevisiae* were the dominating yeasts in bakery rye sourdoughs from Finland, whereas *S. cerevisiae* dominated in rye sourdoughs used for home baking (57). A later study showed that the yeasts isolated from rye bakery sourdoughs in Finland were similar to *C. milleri* (55). Wlodarczyk (25) found that *S. cerevisiae* accounted for 99% of all yeasts found in starters from three industrial rye bread bakeries in Poland, whereas the sourdoughs from the smaller bakeries contained a wider range of yeast strains.

Traditional Portuguese sourdoughs prepared from maize and rye were dominated by *S. cerevisiae* and *Torulaspora delbrueckii* (58), and *S. cerevisiae* and *C. pelliculosa* (28), respectively. *S. cerevisiae* was also the dominating yeast in wheat sourdoughs from Italy, followed by *S. exiguous*, *C. krusei*, *P. norvegensis,* and *Hansenula anomala* (59). Addition of baker's yeast is widely used in some Italian wheat sourdoughs (7), whereas many sourdoughs are also prepared without sourdoughs (60).

The yeasts present in sourdoughs are generally acid-tolerant. Strains of baker's yeast *S. cerevisiae* have poor tolerance of acetic acid in sourdoughs (61), whereas strains of *S. cerevisiae* isolated from sourdoughs can grow on MYGP broth acidified with acetic acid to pH 3.5 (37).

C. Microbial Interactions

The high stability of sourdoughs used for a longer period might be caused by production of inhibitory substances (41), but also microbial interaction between the LAB and the sourdough yeasts are of importance. Several sourdough LAB produce inhibitory substances against spoiling microorganisms. These compounds are organic acids—in particular, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, and diacetyl (62). The inhibition, how-

ever, can also be caused by bacteriocins that are low molecule-mass peptides, or proteins, with a bactericidal or bacteriostatic mode of action, in particular against closely related species (41).

Microbial interaction was early demonstrated for the Sanfrancisco sourdough. The sourdough yeast *T. holmii* (*C. millery*) does not assimilate maltose (34,63), whereas *L. sanfrancisco* hydrolyzes maltose and excretes one of the glucose molecules to be used for the sourdough yeast (64). The glucose uptake of the yeast cell can induce an outflow of amino acids, and this liberation of amino acids has made growth of *L. sanfranciscensis* possible even in a medium initially deficient in essential amino acids (65). Several LAB increase the acidification of sourdoughs when the sourdough yeasts *T. holmii* or *S. cerevisiae* are added (63,66). However, LAB might also multiply more slowly and decrease the production of acids in mixtures with yeasts (67).

A real risk of bacteriophage contamination of sourdoughs exists because bacteriophages with activity against *L. fermentum* have been isolated from an Italian sourdough (68).

IV. TECHNOLOGICAL ASPECTS

A. Production of Sourdough

Sourdough can be made with variations in the following parameters: flour type—wheat/rye, flour extraction rate, flour/water ratio, temperature, time and amount of starter. Sourdough can also be made in one to three steps. The one-stage process is the basic way to make a sourdough and is widely used. Two- and three-step sourdoughs have traditionally been used in rye bread production in many German bakeries (21). Industrialization in bakeries has also included sourdough production, where the time-consuming multiple-stage processes have changed to the work-saving one-stage process. Traditional rye sourdoughs have often been based on firm sourdoughs, but in automated large-scale bakeries, firm sourdoughs are difficult to handle, and they have been replaced by pumpable semifluid to fluid sourdoughs that are suitable for automated fermentation systems. Today, continuous fermentation plants are used in many bakeries in Europe, and they are described in detail in Chapter 42. The following deals with how sourdough fermentation can be influenced by the flour type, flour extraction rate, fermentation temperature, water content in sourdough, and by the amount of added ripe sourdough.

B. Flour Type

The flour in the sourdough is the substrate for the fermenting microorganisms. Wheat and rye flour are mostly used for sourdough making, but maize flour can also be used (28,69). The amount of fermentable carbohydrates in the flour varies with the type of cereal, but in particular with the activity of endogenous enzymes in the flour. The activities of amylases, xylanases, and peptidases are important for liberation of the fermentable low-molecular-weight carbohydrates and amino acids. In the dough stage, the α-amylase cannot degrade intact starch granules, but some granules are damaged during the milling process and may be partly degraded in the dough.

Starch is generally not degraded by LAB, and the content of fermentable mono- and disaccharides in rye flour can reach 5%, with maltose (3%) as the main part (70). Savola found that this content of free sugars decreased by 3% during sourdough fermentation.

However, Henry and Saini (71) found only small amounts of low-molecular-weight sugars in rye (0.7% sucrose and $<0.1\%$ of glucose, fructose, raffinose, and stachylose). The content of pentosans (arabinoxylans) in rye flour is high (6.5–12.2%) (72) compared to wheat flour (2–3%) (73), and they can be degraded to the pentoses xylose and arabinose by the corresponding enzymes during the bread-making processes (74).

The content of fermentable carbohydrates in wheat flour is 1–2% (67,75). The content of maltose increased during the sourdough fermentation from 1.5 to 2.4%, and the content of fructose from 0.05 to 0.45% in a sourdough fermented with *Lc mesenteroides* (75). The content of glucose was unchanged at the level of 0.17% as a result of a balance between bacterial consumption and hydrolysis by the enzymatic activity. No sucrose was detected in the samples, so the increase in fructose could not be caused by yeast invertase.

Most *Lactobacillus* isolated from sourdoughs are nonamylolytic, but amylolytic strains have been isolated from African fermented cereal products made from maize such as ogi, mawé, and kunu-zakki (76).

The extraction rate of the flour is one of the most important factors for determining the character of sourdough (77,78). With a high extraction rate (80–100%), the content of nutrients such as B vitamins and minerals increases compared to low extraction rate flour (65–75%), as does the buffering capacity of the flour primarily due to the phytic acid from the aleurone layer of the cereals. These factors can stimulate the growth and biochemical activity of the microflora in the sourdough, followed by a higher production of acids and flavor compounds. Rye flours have a generally higher extraction rate than wheat flours.

A linear relationship between ash content and TTA was found in wheat sourdough. The final TTA in sourdoughs made from wholemeal flour (ash 1.5%) was almost double the value compared to sourdoughs made from straight-grade flour (ash 0.55%), and the final pH was reached in less time in sourdoughs made from the low extraction flours (3).

C. Water Content

The water content in the sourdough determines the firmness of sourdoughs, and it can be expressed as the dough yield (DY), which is the amount of sourdough in kg per 100 kg flour. DY varies from 150 in firm sourdoughs to 300 in fluid sourdoughs. The development in TTA is lower in fluid rye sourdoughs compared to firm sourdoughs, but if the acidity is measured per gram dry matter, it will be lower in firm sourdoughs (21,36). This indicates that the nutrients are better used by the LAB in fluid sourdoughs compared to firm sourdoughs. The production of lactic acid is not influenced by the DY, whereas the production of acetic acid is generally lower in fluid sourdoughs (21,36). The water content in sourdoughs influences the acidification of the dough more than the temperature (79).

The content of LAB was not influenced by the firmness of rye sourdoughs, whereas the yeast propagation was low in the firm sourdoughs with levels below10^3 CFU/g in six of the seven sourdoughs (36). However, a surface layer of yeast cells was seen on the firm sourdough fermented with *L. plantarum*.

D. Temperature

The temperature of the sourdough is influenced by the temperature of the flour, the water, and the mother sponge, and it is often adjusted/regulated by the water temperature. In practice, the temperature increases 6 to 8°C during fermentation on an industrial scale if the temperature is not thermostatically regulated, so it is important that the temperature

of the water is not too high. The temperature of the sourdough greatly influences the microbial propagation and production of acids, as the optimal temperature for growth and acidification varies for the different species of LAB. Spicher (80) found that the lowest generation time was 20 min for *L. brevis* at 35°C and *L. plantarum* at 40 °C, and 60 min for *L. fructivorans* at 30°C and *L. fermentum* at 40 °C. Changes in the fermentation temperature from the optimal conditions increased the generation time considerably, and the generation time for *L. fermentum* was prolonged to 120 min at 40°C and 140 min at 25°C.

The optimum temperature for growth of the LAB is close to the optimal temperature for acid production, and most LAB have temperature optima between 30 and 35°C (21). In general, the final pH is reached more quickly at higher temperatures (30–35°C) compared to lower temperatures (20–25°C) (21,79,81). Some species, mostly heterofermentative, can grow below 15°C, such as *L. farciminis*, *L. plantarum*, *L. rhamnosus*, *L. brevis*, *L. fructivorans*, *Lb sanfranciscensi*. The highest temperature for growth is between 45 and 55°C, and most species that can tolerate high temperatures are homofermentative, such as *L. acidophilus, L. amylovorus*, and *L. delbrueckii*. However, also the heterofermentative species of *L. pontis*, *L rhamnosus*, *L. fermentum*, and *L. reuteri* can grow above 45°C (45).

The optimum temperature for growth of sourdough yeasts has not been intensively investigated, but it seems to be lower than for the LAB. The optimum temperature for growth of *C. milleri* was determined to be 27°C (44); *C. milleri* and *S. exiguous* do not grow at temperatures above 35°C (56). The minimum temperatures for growth of LAB and yeast are important when sourdoughs are stored by cooling because the sourdough should not develop during the storage. The minimum temperature for growth of most sourdough yeasts has been found to be 8°C (55).

The content of acids produced in sourdough increases with increased fermentation temperature due to higher production of lactic acid, whereas the production of acetic acid is only negligibly influenced by the temperature (21,81). This confirms the general rule that the relative content of acetic acid is higher in cold sourdoughs compared to warmer sourdoughs (21).

Investigation of the influence of the fermentation temperature on the production of flavor compounds in rye sourdoughs showed that the starter cultures themselves produced few volatile compounds, whereas the production of iso-alcohols and ethyl acetate increased considerably with higher temperature in sourdoughs fermented with homofermentative LAB due to activity by the propagating yeasts (81).

E. Amount of Mother Sponge

The amount of mother sponge to be mixed with flour and water for a new sourdough should be so high that the content of LAB in the sourdough is able quickly to decrease the pH to inhibit the growth of the gram-negative bacteria in the flour. The amount of mother sponge influences the pH-lowering capacity in a sourdough, as low pH is reached more quickly when the amount of added mother sponge is high (79). However, higher levels of acids are produced when a lower amount of mother sponge is added, as the fermentation time is longer before the pH drops to the critical pH level (20). The recommended amount of mother sponge is generally 10–20% for both rye and wheat sourdoughs (21,79). The Sanfrancisco sourdough is rebuilt every 8 hr or at least two to three times a day, 7 days a week. The amount of mother sponge used in preparing a new sourdough is 25–40% of the sourdough (15). This high amount of mother sponge makes the sourdough very stable, and this sourdough has been continued for more than a century.

V. DOUGH PROPERTIES AND BREAD QUALITY

A. Dough Properties and Bread Texture

1. Wheat Dough and Bread

Incorporation of sourdoughs in wheat bread making influences the gluten proteins and the viscoelastic behaviour of doughs due to the drop in pH value caused by the organic acids produced. Several investigations have shown that the addition of acid to wheat dough decreased the dough stability during mixing, and the acidified doughs became considerably softer than a nonacidified control dough (82–84).

Dough stability was also decreased when it was prepared with the addition of sourdough (85,86). The dough consistency was unchanged when the sourdough was fermented by a heterofermentative culture and softer if a homofermentative culture was used (86). Proteolytic breakdown of proteins was enhanced at low pH during fermentation of wheat dough, and major effects were attributed to changes in pH rather than to microbial proteolytic activity from the sourdough (87).

In spite of the decreased stability in doughs with added sourdough, increased bread volume is reported for bread containing up to 20% sourdough (31,86). The crumb structure of bread containing up to 20% sourdough has been comparable to standard bread without sourdough, whereas inferior crumb structure was observed in bread containing 40% sourdough (88).

2. Rye Dough and Bread

The main component of rye and wheat is starch, and its content has a crucial influence on the bread texture. It becomes sticky and pasty if the starch is degraded during the bread making due to too high activity of amylases. This problem is greater in rye bread making than for wheat bread, as the activity of the sprout-induced enzyme α-amylase is highest in rye (89). This is caused by rainy summers in the rye-growing area. Furthermore, the period from harvest to possible sprouting is extremely short for rye; it can even sprout in the fields (89). One of the main functions of sourdough in rye bread making is inactivation of the α-amylase activity, and a general rule in bakeries is to add a larger amount of sourdough when the activity of enzymes in the flour is high. Bread with a rye content of more than 20% normally requires the addition of sourdough to prevent degradation of starch (88,90). Rye starch begins to swell as low as 52°C and subsequently the α-amylase can degrade the starch until it will be heat-inactivated at 80°C (91). Rye α-amylase has a pH optimum at pH 5.5 (92), and the activity is totally inactivated in sourdough at pH below 4. Wassermann and Dörfner (93) found that the viscosity of rye doughs (rye flour and water) was lowest at pH 5.

The activity of α-amylase is not only reduced considerably in the sourdough, but also in the rye dough with added sourdough. The activity of α-amylase was totally inactivated in an imitated sourdough acidified to pH 3.5 (TTA 32) by lactic and acetic acid (94). The activity of α-amylase in the bread dough after resting (pH 4.5), with 20% sourdough added, was about half the activity in the flour.

Pentosans (arabinoxylans) play a key role in the viscosity of rye doughs due to high water-binding capacity. The viscosity of sourdoughs decreases during the sourdough fermentation due to the activity of the pentosan-degrading enzymes at the beginning of the fermentation. However, those enzymes are inactivated in the fermented sourdough (94). Rye proteins are different from wheat proteins, as they do not form gluten structure.

Kratochvil and Holas (95) found that proteolytic activity in rye sourdough was caused by enzymes from the flour.

B. Flavor and Taste

The flavor of bread crumb depends mainly on the flour type and the enzymatic reactions taking place due to yeast and sourdough fermentations, whereas the flavor of bread crust is more influenced by the thermal reactions during the baking process. Including sourdough in the bread recipe is recommended for a more aromatic bread flavor (31,96) and sourdough bread has a higher content of volatile compounds (31,39,97–99) and higher scores in sensory tests (31,100,101). The content of volatile compounds produced during sourdough fermentation depends on the flour type (wheat, rye, maize), the extraction rate of the flour, the fermentation temperature, the water content in the sourdough, and the microorganisms in the sourdough. Generally, the LAB in the sourdough are mostly responsible for the acidification of the dough, and the sourdough yeasts for the production of flavor compounds. Factors that favor the propagation of yeasts will also result in higher content of yeast fermentation products.

The extraction rate of the flour and the water content in the sourdough mostly influences the acidification of the sourdough. Higher extraction rate of the flour results in higher production of lactic and acetic acid (38,102), however, sourdoughs fermented with heterofermentative cultures have much higher content of ethyl acetate (38). The production of acids calculated per gram dry matter is higher in fluid sourdoughs than in firm sourdoughs. Higher water content in the sourdough and increased fermentation temperature result in higher propagation of yeasts and in higher content of iso-alcohols (36,81,102).

Sourdoughs fermented with heterofermentative LAB have, aside from much higher content of acetic acid and ethanol, a higher content of ethylacetate and ethyl-hexanoate compared to sourdoughs fermented with homofermentative LAB, which have higher contents of diacetyl and some other carbonyls (36,81,102,103). The production of acetic acid in sourdoughs can be increased in heterofermentative cultures with the addition of fructose as a hydrogen acceptor (102,104). When sourdough yeasts are added in the preparation of the sourdough, the production of ethanol, iso-alcohols, esters, and diacetyl increase considerably (37,103).

In sourdough bread, the content of esters is very low compared to the corresponding sourdoughs (31,39). Sensory evaluation of rye bread crumb shows that the most intense and breadlike flavor is associated with 2-propanone, 3-methyl-butanal, benzylalcohol, and 2-phenylethanol (39). However, vanillin, 2,3-butandione, 3-hydroxy-4,5-dimethyl-furanone, and methylbutanoic acids also contribute to the overall crumb flavor (98). The perceived taste of salt is enhanced in sourdough rye bread compared to wheat bread, so less salt can be added in sourdough rye bread (105).

Sensory evaluation of wheat bread crumb showed that bread made with sourdough fermented with the heterofermentative *L. sanfranciscensis* had a pleasantly mild, sour odor and taste. Bread fermented with *L. plantarum* had an unpleasant metallic sour taste, but when the sourdough was also supplemented with the sourdough yeast *S. cerevisiae,* the bread acquired a more aromatic bread flavor. That bread had a higher content of methylbutanol, methylpropanoic acids, and 2-phenylethanol, which may, in part, cause the more aromatic flavor (31). Mixed cultures with both LAB and yeast are recommended for an aromatic and pleasant sourdough bread flavor (31,103,106). A lexicon for description of the flavor of wheat sourdough bread has been developed (107).

C. Longer Shelf Life

During storage of bread, several different physical and microbiological changes occur, lowering the quality of bread. The bread crumb becomes hard, the bread crust changes from crispy to leathery, and the characteristic and favorable bread flavor disappears. All these changes are characterized as the staling process. Within few days the bread might be spoiled due to contamination and growth of molds on the surface or development of rope in the bread crumb caused by *Bacillus* spp. Addition of sourdough in the bread recipe can be used to retard the staling process of the bread, prevent the bread against ropiness and prolong the mold-free period. Sourdough addition is the most promising procedure to preserve bread from spoilage, since it is in agreement with the consumer demand for natural and additive-free food products.

1. Anti-Mold Activity of Sourdough Bread

Mold is the most frequent cause of bread spoilage. Addition of sourdough in the bread recipe increases the mold-free period for rye bread (21,32) and wheat bread (78,108). The length of mold-free period was prolonged from 4 days in wheat bread to 6 to 8 days in sourdough bread (78). No correlation was found between pH and bread shelf life (108).

The mold-free period was prolonged 1 to 3 days in slices of sourdough rye bread inoculated with *Aspergillus glaucus* when the sourdough was fermented with heterofermentative LAB compared to homofermentative LAB, or bread without addition of sourdough (32). The antimicrobial effect of the heterofermentative LAB was supposed to be the result of their production of acetic acid.

Using agar-well-diffusion assay, 232 strains of sourdough LAB belonging to nine different species were screened for production of anti-mold substances against *Aspergillus niger*, *Fusarium graminearum*, *Penicillium expansum*, and *Monilia sitophila* (109). The anti-mold activity varied very much among the strains and was mainly detected within obligately heterofermentative LAB. *L. sanfrancisensis* had the largest spectrum of anti-mold activity. Not only the acetic acid had inhibitory effect, but the LAB produced also formic, propionic, butyric, n-valeric, and caproic acid, and a mixture thereof was responsible for the anti-mold effect.

2. Prevention of Rope Spoilage

Ropiness is spoilage of wheat bread noticed as an unpleasant odor similar to that of overripe melons, followed by the occurrence of a discoloured sticky bread crumb and sticky threads that can be pulled from the crumb. This bread spoilage is caused by heat-resistant strains of *Bacillus* and occurs particularly in summer when the climate favors growth of the bacteria. It is mainly caused by *Bacillus subtilis,* formerly referred to as *B. mesentericus*, because the heat-resistant spores can survive the baking process, sporulate, and multiply in the baked bread. The rope symptoms can be recognized when the level of *Bacillus* in bread crumb is 10^8 bacillus/g (110). Its incidence has increased during the past decade, presumably because most bread is now produced without preservatives and often with the addition of raw materials such as oat products, wheat bran, and sunflower seed with a high contamination level of *Bacillus* spores (110). Even a low level of the heat-resistant spores (10^1–10^2 bacillus/g) in raw materials resulted in a level of 10^7 bacillus/g bread in 2 days.

One potential way to prevent development of rope is to include sourdough in the bread recipe. Addition of 10% sourdough inhibited the natural *Bacillus* contaminants in wheat dough, but it was insufficient to inhibit the bacillus strains inoculated at a level of

10^6 spores/g (40). Addition of 15% sourdough was more efficient as the strains of rope-producing *Bacillus* were effectively inhibited by sourdough fermented by strains of *L. sanfranciscensis*, *L. brevis*, *L. maltaromicus*, or by three different strains of *L. plantarum*. In this investigation, *B. subtilis* tended to be inhibited if the TTA value in the sourdough was more than 10 and when the pH of the bread crumb was below 4.8. Röcken (29) demonstrated that sourdough effectively decreased the heat resistance (D_{97}-value) of a rope-producing strain of *Bacillus*. He found that the heat resistance was reduced from 143 min without the addition of sourdough to 5.9 min and 6.9 min with the addition of 10% and 20% sourdough, respectively.

3. Bread Firmness and Staling Rate

Bread becomes firmer during storage, and retrogradation of starch towards a more crystalline form is considered to be the primary cause of this bread staling. Several sourdoughs have been investigated for their potential effect on delaying the development of bread firmness and staling rate of wheat bread, but most investigations did not find any influence on staling rate by the sourdough compared to yeast- and sponge-leavened bread (108,111). However, delayed staling rate has been observed in sourdough bread (112). The rate of starch retrogradation was not influenced if the acidification was rather low, whereas a standard sourdough (*L. sanfranciscensis* 57, *L. plantarum* 13, *S. cerevisiae* 141) was able to retard the staling rate. The staling rate was mostly influenced if the starter culture had amylolytic activity (*L. amylovorus* or a genetic modified strain, *L. sanfranciscensis CB1 Amy*).

In some investigations, the addition of sourdough resulted in lower bread firming. However, sourdough wheat bread has higher bread volume (31,86,88) and the measured resistance will thus be lower.

VI. NUTRITIONAL VALUE

The addition of sourdough to the bread recipe has a positive influence on the nutritive value of the bread because the minerals become bioavailable (113), and blood glucose and insulin responses are lowered after eating sourdough bread compared to wheat bread (114).

A. Reduced Phytate Content by Sourdough

Whole-meal cereals are good sources of minerals such as K, P, Mg, Fe, and Zn; but without treatment, the bioavailability is poor for minerals stored as phytate, an insoluble complex with phytic acid (myoinositol hexa-phosphoric acid, IP6). The content of phytate is 6 mg/g rye grain (115), 3–4 mg/g in flour of soft wheat and 9 mg/g in hard wheat flour (116). Phytate accounts for more than 70% of the total phosphorus in cereals, and it can be degraded during the bread-making process due to the activity of endogenous phytase and thus liberate the bound minerals when the ester-bound phosphoric acids are hydrolyzed. The pH-optimum of rye phytase is found to be at pH 6.0 (115).

Sourdough fermentation has been shown to be more efficient than yeast fermentation in reducing the phytate content in whole bread (−62% and −38% respectively) (113). The prolonged fermentation with sourdough enhanced acidification and led to increased solubility of Mg and P. Five different strains of LAB isolated from sourdoughs have been

tested for their ability to degrade phytic acid, but no difference was observed among the strains in the levels of phytic acid hydrolyses (117).

B. Reduced Glycemic Response with Sourdough Bread

Conventional wheat bread products are rapidly digested and absorbed, thus giving rise to high blood glucose and insulin responses. Eating wholemeal sourdough bread resulted in both lowered blood glucose and insulin response compared with eating wholemeal bread made without sourdough (118). This nutritional positive effect was possibly due to a reduced gastric emptying rate caused by the lactic acid produced during the sourdough fermentation (114).

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Fermented Doughs in Bread Production

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I. INTRODUCTION

Several thousand years ago, dough was already being fermented to produce loaves and similar bakery products with a porous crumb, a significant step forward in baking methods (1,2). This method of baking was based on gas formation caused by fermentation, which produced doughs with a frothy consistency. Figure 1 shows that fermentation is the key process for bread production. As pointed out in Table 1, the leavening of dough by gas formation is accompanied by microbial and biochemical conversion of dough constituents, which results from the action of microorganisms added to the dough and/or which proliferate in the dough and enzymes present in the flour. These conversions require specific conditions from which the working parameters and the technical realization of the fermentation processes result.

Gas formation is the most important effect of fermentation because it creates the foam-like structure of dough that is the prerequisite for a rapid heat flow through the doughs. Heat applied to doughs permits the gas and water vapor to be expelled from the dough while gelatinization of the starch causes the frothy structure of the dough to set, thus producing the crumb. Before dough was leavened by fermentation, it was only possible to bake flat pieces of dough. The thinness of the dough allowed heat to be transferred so rapidly that a loose, coarse dough structure resulted because of water evaporation.

Dough fermentation is based on metabolic activities of yeast and lactic acid bacteria. The ability of these microorganisms to anaerobically produce functional metabolites in doughs, made from flours of wheat and rye milling products varying in composition, is used to achieve the effects shown in Table 2. These primary, secondary, and tertiary effects have been classified according to kinds and functions in bread baking. The individual effects and their interdependence in bakery products have already been comprehensively described (3–6). By controlling the processing parameters of microbiological fermentations and those of technical methods of dough preparation, it is possible to affect microorganisms and enzyme activities present in the dough as required for the particular dough characteristics, such as volume, consistency, and metabolite formation.

The action of yeast, lactic acid bacteria, and enzymes present in doughs can be assisted by adding enzyme preparations (malt flour, α-amylases, pentosanases) and other ingredients (sugar, emulsifiers, ascorbic acid) to the recipes. It should also be mentioned that besides assisting the microbial and enzymic action by recipe modulation, it is also possible to

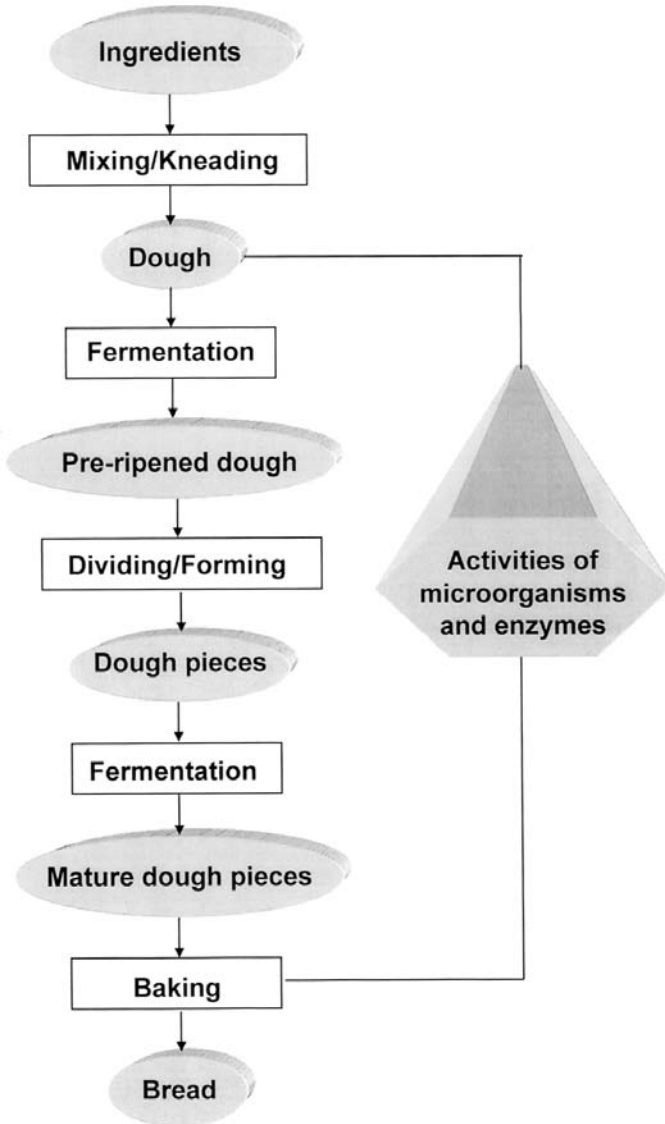


Figure 1 Simplified diagram for bread production from fermented doughs.

improve the performance of the microorganisms by genetic modification (7–9). The dough-making performance of flours can also be improved by genetically modifying the viscoelastic properties of wheat varieties (10,11).

II. PURPOSES AND AIMS OF DOUGH FERMENTATION

The various methods of dough fermentation pursue the same goals which are emphasized differently to achieve the particular properties of the various bakery products. The different

Table 1 Conversion of Dough Constituents by Microorganisms and Enzymes

Constituent	Yeast	Lactobacilli	Enzymes
Starch	–	–	α- and β-Amylases
Dextrins	–	–	α- and β-Amylases
Maltose	+	+	–
Sucrose	+	+	–
Glucose	+	+	–
Fructose	+	+	–
Pentosans	–	–	Pentosanases
Proteins	+	+	Proteases

degrees of emphasis in fermentation concern the consistency of the fermented dough and the effects obtained by fermentation as described and evaluated in Table 3. From this evaluation, objectives can be recognized that have led to the development of widely differing processing techniques to cover a wide variety of bakery products over a range of processing and marketing conditions. To some extent, the availability and the types of raw material, in particular the types of flour, need to be considered.

In order to illustrate the differences in the demands on fermentation processes, it has to be pointed out that, for example:

Dough-formation characteristics of European soft wheat flours differ from those of American hard wheat flours.
Production of breads and rolls from mixtures of wheat and rye milling products require both yeast and sourdough fermentations.
Large assortments of bakery products generally call for versatile equipment.
Competition in the marketplace leads to division of labor and specialization.
Statutory requirements, such as legislation regarding working hours, mean that equipment cannot be used as effectively as it might.

However, there is a general trend toward simplifying, curtailing, and automating processes involving fermentation. This enables their use in computer-aided process control systems and computer-integrated manufacture. In this connection, economic pressures force bakeries to be more productive and at the same time retain or improve the quality of bakery products by using the latest technology. This is a difficult problem with regard to fermentation required for dough preparation because it presupposes control of a complex mass flow that depends to a large extent on biological laws. Control must therefore be based

Table 2 Classification of the Effects of Dough Fermentations According to Kinds and Functions in Bread Baking

Effects	Kind	Function
Primary	Formation of functional metabolites (Carbon dioxide, ethanol, lactic acid, acetic acid)	Volume, texture, taste, shelf life
Secondary	Degradation of high molecular substances (Pentosans, α-glucans, proteins)	Consistency, texture, shelf life
Tertiary	Formation of improving metabolites (Flavor precursors: Maillard reaction components)	Flavor, color

Table 3 Description of the Consistency of Fermented Doughs and Evaluation of the Purposes of Dough Fermentations

	Consistency of fermented dough		Effects		
Baked product	Viscous	Liquid	Primary[a]	Secondary[b]	Tertiary[c]
Baguette		•	+	+	+ +
Cracker	•			+ +	
Hamburger bun		•	+ +	+ +	+
Toast	•	•	+ +	+	+
Rye bread	•	•	+ +	+ +	+
Wheat/rye bread	•	•	+ +	+	+

[a] Functional metabolites (carbon dioxide, organic acids) cause foam-like structure of doughs.
[b] High-molecular substances (proteins, pentosans) influence dough consistency.
[c] Improving metabolites (alcohols, aldehydes) form precursors for bread flavor.

on foresighted planning covering a period of 1–24 hr. All factors, such as temperature, cell count, initial activity, and growth rate, that affect the progressing fermentation must be mastered effectively because they are decisive for the quality characteristics of the dough from which the final quality of the bakery products is dependent.

A certain level of knowledge of the fundamentals of making gas-leavened doughs, such as gas formation in dough, its dependence on the dough ingredients, and the mechanics of dough preparation, is required if the various aspects of making doughs with fermented dough referred to above are to be taken into account.

III. FOAMING OF DOUGHS

Frothy doughs can be produced by either the formation or dispersion of gas bubbles in doughs. Gases can be formed biologically, chemically, or physically. The gases are dispersed throughout the doughs in the form of bubbles to produce fine-pored foams. This applies particularly to the formation of CO_2. The fineness of the pores is a result of the dough production process during which—depending on the functional properties of the constituents of the flours (e.g., viscoelasticity of the wheat gluten, water-binding capacity of pentosans, and foam-forming capacity of rye proteins)—air is incorporated in the dough and dispersed throughout it in the form of very fine bubbles when flour and water are mixed and kneaded. The entrapped air bubbles act as nuclei that absorb the CO_2 formed, making the dough rise and resulting in a spongy structure (12,13). The considerable volumetric expansion that occurs—exceeding 1:6 for wheat doughs and 1:3 for rye doughs—leads to a corresponding decrease in the density of the doughs. Gas formation by fermentation is accompanied by a number of other fermentative reactions—for example, enzyme secretion by the microbial cell mass, and formation of intermediary metabolites. These fermentative reactions not only result in the formation of metabolites from the constituents of the ingredients used in the recipes but also in changes in the viscous properties of the doughs and the formation of precursors of aroma components brought about by the enzymic conversion of the constituents and intermediary metabolites. The characteristic properties of bread depend on such biological-enzymic reactions and distinguish it from all other bakery

products, such as a particular rye crispbread (14) that is not made by a biological-enzymic method. This particular rye crispbread is mentioned here because it is an example of a frothy dough made by mechanically dispersing gas in a cooled flour-water suspension under the action of shear flows. The retention and distribution of gas in this case depends on the specific foam-forming capacity characteristic to particular rye protein fractions (15).

IV. GAS RETENTION OF DOUGHS

The gases produced in, or incorporated into, the dough and entrapped in it in the form of gas bubbles require the development of pores. The formation of extensible pore walls, both in wheat and rye doughs, depends on certain proteins present in the flour that exhibit specific film-forming properties. Such properties are particularly pronounced in the gluten proteins (gliadin and glutenin) found in wheat flour. When mixed with water under the action of mechanical energy, wheat gluten forms a viscoelastic mass that encloses all other constituents of doughs, primarily starch, in a network. The latter can be two-dimensionally stretched to films largely impermeable to gas. These physical properties of gluten enable it to entrap gases as bubbles, forming a porous, three-dimensionally extending structure. The structure sets to form the crumb of the bakery products when the starch gelatinizes and the gluten coagulates under the action of heat during baking.

Figure 2 shows gluten protein particles isolated from wheat flour that have been converted into a viscoelastic mass by moistening and kneading. When the residue was freeze-dried and examined using a scanning electron microscope, it was possible to identify a membranelike network that corresponds to the structure that is formed by the gluten proteins and is required for the development of the membrane and network in actual doughs. The formation of the structure depends on the amino acid sequences of the gluten proteins and their chemical reactivity. In this connection, it was possible to prove experimentally that the disulfide bonds of wheat gluten contribute substantially to the characteristics of the dough and thus to its baking performance (16,17). The molecular composition of wheat gluten is now also known to a large extent (18). In this context, it is interesting to note that the relative composition of gluten in respect to its molecular subfractions gliadin and glutenin has a crucial influence on its stretching properties, which are responsible for providing the volume in bakery products (19). As regards the baking volume, flours from different wheat varieties thus exhibit differences in volume-forming capacity. The latter depends on an optimal development of the viscoelasticity of the dough by kneading. The mechanical energy input by kneaders necessary for an optimal viscoelasticity is in the range of 10–12 Wh/kg dough (20).

The proteins present in rye flours do not possess the same viscous properties as wheat gluten even though rye flour protein contains fractions that, owing to their foam-forming properties, are suited to incorporate gases in doughs in the form of bubbles (14). However, such doughs are considerably less firm than wheat flour doughs due to the lack of an elastic protein fraction in their foam structure. This difference between wheat and rye flour doughs is evident in the surface structure of each type of dough, as shown in Fig. 3. The wheat flour dough has a smooth structure, whereas the structure of the rye flour dough is loose and fractured. Unlike wheat flour doughs, rye flour doughs therefore tend to flow so that they generally result in bakery products that are flatter than those made of wheat flour dough. The difference in gas retention between wheat and rye flour doughs results in the shown large differences between the volumes of the breads baked in both cases from the same dough weight.

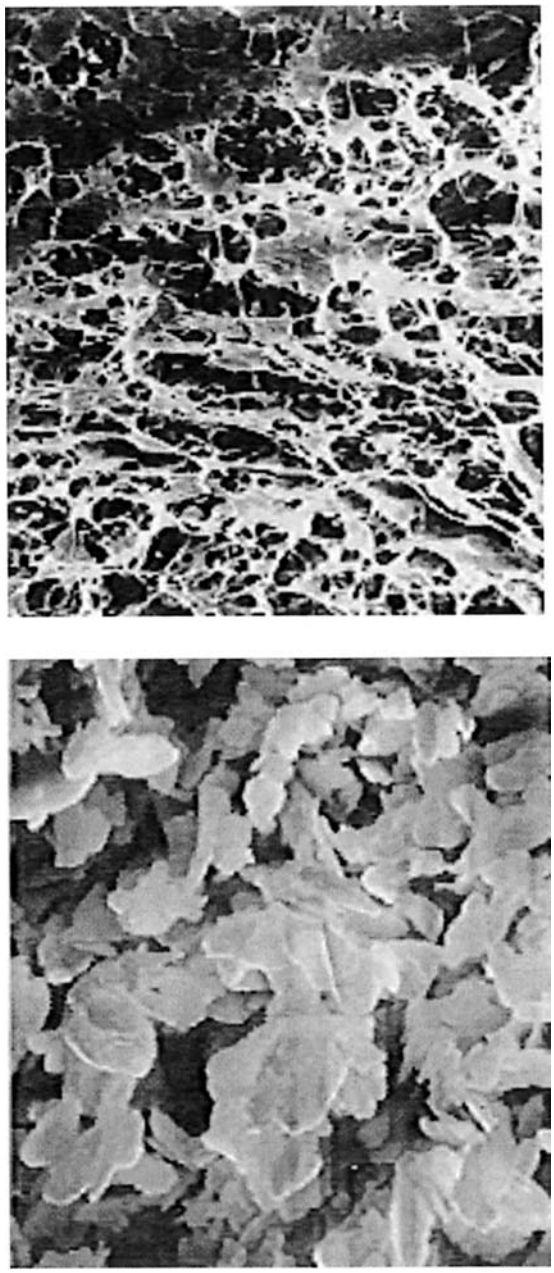


Figure 2 SEM of gluten protein particles extracted from wheat flour (bottom) and of freeze-dried gluten-dough (top).

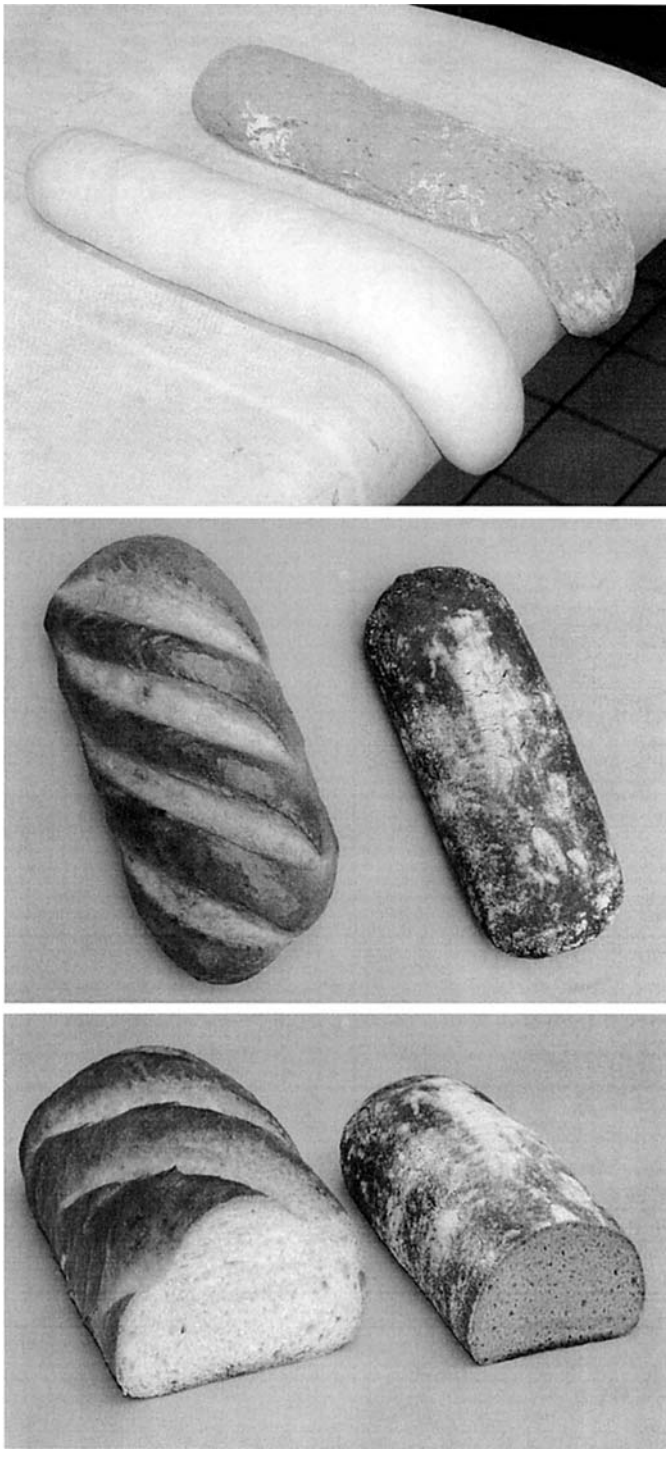


Figure 3 Doughs and breads made from wheat and rye flour.

V. TECHNOLOGICAL ASPECTS OF DOUGH FERMENTATION

Very good accounts of the fermentation and preparation of doughs, describing the large variation in time needed to produce them while taking into account the mechanical and chemical methods of preparing dough, are already provided by textbooks (20–22). It can be deduced from this literature that all these processes have both advantages and disadvantages in the production of certain bakery products. For example, the short preparation time (2 hr) required for wheat flour doughs without bulk fermentation as compared with that required for wheat doughs with bulk fermentation (4–7 hr) is an advantage. However, the rapid formation of gas caused by the high proportion of yeast used means that it can be difficult to keep to the processing schedule required for this kind of dough preparation. A very tight schedule is required for dough preparation because of the rapid increase in volume. Keeping to it is crucial because the final stage of fermentation has a decisive influence on the volume and shape of the bakery products.

The variations in fermentation time for sourdoughs (3–48 hr) in which rye milling products are predominantly used is even greater than that for yeast doughs. All microbiological sourdough fermentations are based on the active proliferation of the sourdough microorganisms. In contrast with this, the ability of yeast to proliferate under anaerobic conditions is exploited in dough fermentation only to the extent that the weak proliferation and growth of the yeast cell during fermentation contributes to reducing the fermentation time required for the development of the dough and the formation of the dough volume.

In sourdough fermentation, proliferation is brought about by a ripe sourdough (alternatively, by one in which metabolic activity has ceased) used as an inoculum (23). A portion of this sourdough is used to make a fresh mix, together with flour and water. All sourdough preparations are bulk fermentations, the sourdoughs acting as preliminary doughs to produce bread doughs. The proportion of inoculum in the sourdough determines the process and method of fermentation, depending on the ripeness of the sourdough required for making bread dough. Based on the ratio of fermented flour to total flour, the inoculum can range between 1 and 50%.

VI. PRELIMINARY DOUGHS

In order to provide a satisfactory account of the principal advances in sourdough production, it is necessary to take a look at the entire system of preparing preliminary doughs and to examine the role of preliminary doughs, in particular sourdoughs, in breadmaking. This requires a means of classifying preliminary doughs that takes into account not only their functional properties but also the technological developments that have been put into practice in different geographical regions of the world.

Preliminary doughs can be subdivided into

Sourdoughs: doughs fermented by lactobacilli and sourdough yeasts
Sponges: doughs fermented by yeast
Mashes: mixtures of kernels and/or milled products with hot or cold water
Bread syrups: enzymatically degraded bread crumbs
Liquid ferments: enzymatically degraded fermented mashes containing yeast and/or lactobacilli

Such preliminary doughs are used to different extents in different parts of the world, and for different purposes. For example, sourdoughs, mashes, and bread syrups are widely

employed in Germany, whereas sponges, sourdoughs, and liquid ferments are common in America and sourdoughs and liquid ferments are used extensively in Russia (24).

Although production and use of sourdoughs to make different types of baked goods has been known since ancient times this is not true of most other preliminary doughs, in particular their most modern types. These modern preliminary doughs have gained in importance as the production of baked goods has become more mechanized and industrialized. Their development is partly due to mechanization and industrialization, yet it also originated in the diversification of the range of bakery products. As developments in technology and diversification of the range of available bakery products have given rise to new challenges in the production of baked goods, it is necessary to examine briefly the different functional properties of the preliminary doughs and the way in which they affect the quality characteristics of the final products.

Essentially, the role of preliminary doughs in making bread and other bakery products consists not only in improving baking performance, crumb structure, digestibility, and chewiness, but also in delaying crumb staling and prolonging shelf life (6). In addition to this, preliminary doughs, like bread syrups colorized by pressure cooking (25) or mashes soaked in hot water, can be used to determine the color and texture of the crumb. Another important function of preliminary doughs consists in lending the final products a specific flavor. Each preliminary dough fulfils these functions to a limited degree only. Therefore, the selection of a specific preliminary dough depends on that quality criterion of the final product that has to be influenced. A combination of several preliminary doughs may be required for certain bakery products. For example, combinations of sourdoughs, mashes, and bread syrups are used in German wholemeal bread varieties (26).

The specific properties of the preliminary doughs are obtained by means of mechanical, fermentative, enzymic, and thermal process steps. The simplest step involves mixing milled products with water and leaving the mixture to swell for a certain period of time. Swelling can be accelerated by heating the mixture in order to promote reactions of the enzymes present in the raw materials, for example (soaked dough, obtained by soaking coarsely ground grain in warm water). The mixture may also be heated to such a degree that some of the starch is gelatinized (hot soaked dough, obtained by soaking coarsely ground grain in hot water). Incipient gelatinization of the starch is essential if the starch is to be degraded to saccharides (glucose, maltose), which is what happens, for instance, when bread syrup is made by adding amylolytic enzymes. Besides enzymic degradation, it is above all the saccharides of the swollen cereal that can be fermented by metabolic activity of lactic acid bacteria and yeasts and produces either sourdoughs or sponges.

Accordingly, preliminary doughs are produced in accordance with a few basic principles comprising swelling (pentosans, proteins), fermentation (microorganisms, enzymes) and gelatinization (starch), each of which can be adapted in a variety of ways. There is thus wide scope for varying the functional properties of preliminary doughs. This applies in particular to the microbiological production of preliminary doughs.

VII. SOURDOUGHS

A number of different sourdoughs and methods of fermenting them have been described in the literature (6,27–33). Common to all sourdoughs is the proliferation of lactic acid bacteria that accompanies the formation of organic acids, predominately lactic acid and acetic acid. The properties of the respective sourdoughs are the result of that metabolic activity, which in turn can be influenced by production conditions.

The way in which microorganisms and the process variables of sourdough fermentation affect the principal properties of sourdough and the subsequent bread quality are illustrated in Fig. 4. The figure shows that the properties of the sourdough are not only affected by the type and number of species of microorganisms but also by the total number of microorganisms and their phases of development in the sourdough. The effect of the microorganisms develops as a result of the metabolic activity, which is coupled with a considerable degree of proliferation. The formation of metabolites is therefore a direct function of the number of microorganisms. Lactic acid bacteria predominate, both as far as the number of species and their total number of metabolic active cells are concerned, although various species of yeast also occur in naturally fermented sourdoughs.

The umbrella term "microorganism flora" is used to denote the different species of microorganisms present in sourdough. In naturally fermented sourdoughs, the development of the composition of the microorganism flora depends on the type of microorganisms with which the substrates are contaminated. The result is a biotope in which the composition of species is in a steady state determined by the generation time of the microorganisms. The generation time depends on the process variables used to produce the sourdough.

The principal process variables are fermentation temperature and time, flour-to-water ratio, and type of flour. The fermentation time is the most important process variable because it directly determines the extent of the metabolic performance of the microorganisms. It therefore governs how sourdough fermentation is integrated into the overall bread-making process. The other process variables mentioned are further mutually dependent parameters that control the development of the properties of the sourdough (e.g., acidity, lactic to acetic acid ratio, viscosity, density).

Substrates, which consist essentially of milled products and water, are converted to sourdough primarily as a result of the reduction in the pH following the formation of organic acids and the simultaneous formation of carbon dioxide. Endogenous enzymes in

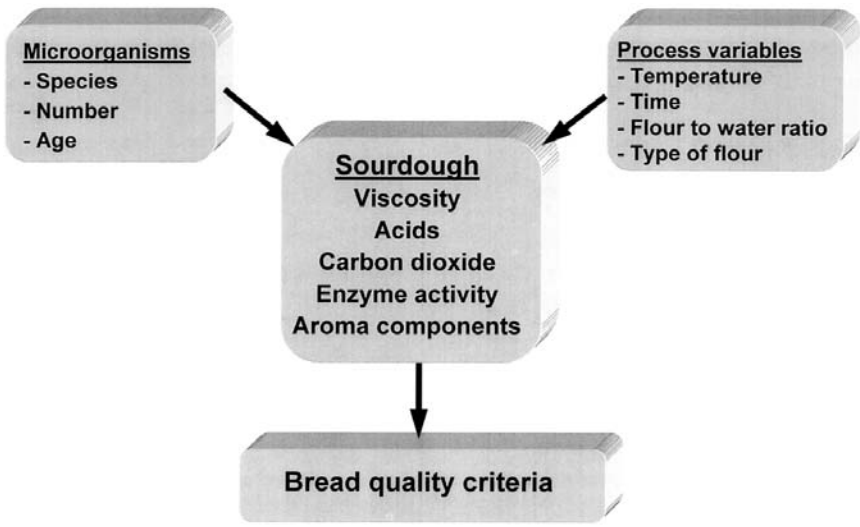


Figure 4 Influence of microorganisms and process variables of sourdough fermentation on bread production.

the milled product are either activated or inactivated during the conversion of the substrate, resulting in the degradation of polymer substances, in particular of nonstarch carbohydrates (e.g., pentosans). Some amino acids and peptides are also released. Some of the products of degradation are digested by the microorganisms; others act as precursors of flavor components (34).

The enzymic degradation of polymer carbohydrates in conjunction with the change in the density of the dough owing to the formation of carbon dioxide results in a marked reduction in the viscosity of the sourdough. As metabolic performance and the accompanying substrate conversion depend on the proliferation of microorganisms, sourdough production generally takes longer than all the other process steps required to make common bread varieties. Production of the well-known San Francisco sourdough bread is an exception, the length of time it takes to produce the San Francisco sourdough corresponding roughly to the time that the dough takes to rise after it has been divided into loaves (35,36).

It is crucial that the properties of the sourdough are transferred to the bread dough and fulfil their functions until the baking process has been concluded. For example, when making bread from rye milled products it is important to reduce pH (below pH 4.6) of the bread dough by adding sourdough in order to inactivate the α-amylase and thus prevent excessive liquefaction of the leavened dough piece as a result of starch degradation during the early stages of heat transfer in the baking process. In addition, the bread dough absorbs more water owing to the extensive swelling of the polymer substances in the sourdough, resulting in an increase in dough water content, with the effect that bread baked from these doughs exhibits an improved freshness and a retarded staling (6). Finally, it should be mentioned that more precursors of flavor components are formed in sourdoughs that are fermented over a long period of time (8 hr) than in those that are produced over a shorter period (2–4 hr) (37).

A. Principles of Sourdough Preparation

This survey of the functions of sourdoughs raises the question as to which fundamental criteria should be applied when adapting sourdough preparation to modern methods of producing baked goods. As already mentioned, the main criterion for the modern-day production of baked goods, whether in small or large bakeries, is how to increase productivity. The greatest advances in productivity have been achieved by mechanizing production processes which requires optimization of product flow schedules during each process step. For sourdoughs, optimization is limited by the laws of microbiology that govern the proliferation of microorganisms and the accompanying metabolic performance.

In bread-making, the growth phases and metabolic performance of the microorganisms are a quantity that can be calculated in a similar way to manpower, which is subject to legislation regarding working hours. This applies, for example, to the way in which working hours are organized, whether in a single shift or several daily shifts. Thus, three shifts per day, 6 days a week, permit uninterrupted production during that period. Sourdough production, being governed by the laws of microbiology, can be fitted into uninterrupted production processes far more easily than into production rhythms comprising only a single shift per day over the same period (38).

However, the aim of both types of production is to make bread of a consistent quality day by day in order to ensure success on the market. This presupposes that the quality of each of the raw materials used does not change and that all sections of the production plant operate uniformly. Achieving this aim is rendered more difficult by the chosen range of bakery products and the subsequent raw material requirement and use of plant. The oper-

ation of small and plant bakeries alike therefore places especially great demands on both internal and external logistics and the data processing required.

The production of sourdough plays an important part in the system of logistics as its use is crucial to the quality of the bread. It must also be borne in mind that a constant bread quality can be achieved only if the sourdough, as one of the main ingredients in recipes, also has uniform quality characteristics. It is therefore necessary to ensure that the most important properties of the ripened sourdough remain constant. Those properties are essentially a constant leavening capacity, characterized by a constant pH and acidity, and a constant flavor, which depends in turn on constant lactic acid and acetic acid contents. In order to maintain these properties, which constitute the quality characteristics of ripened sourdough, the process variables must be controlled in such a way that reliability of production is ensured.

Both in small bakeries and in plants it used to be possible to ensure constant leavening capacity and flavor by employing methods that had been developed by bakers specifically with that purpose in mind. However, when it came to increasing productivity, these methods were shown to lack the flexibility required if the use of manpower or the product flow were to be rationalized. The crucial breakthrough as regards technical advances in the production and use of sourdough did not come until it was realized that it is possible to produce sourdoughs on the basis of high dough yields (200%).

The main difference between modern and traditional methods, such as the method of producing San Francisco sourdough (wheat sourdough) or German three-stage sourdough (rye sourdough) is thus the viscosity. Unlike classic high-viscosity sourdoughs, low-viscosity sourdoughs with high dough yields are pumpable, which is a considerable advantage as regards technological developments (Fig. 5). This paved the way for the continuous production of sourdough and for storing sourdough that is ready for use in both daily and weekly production rhythms.

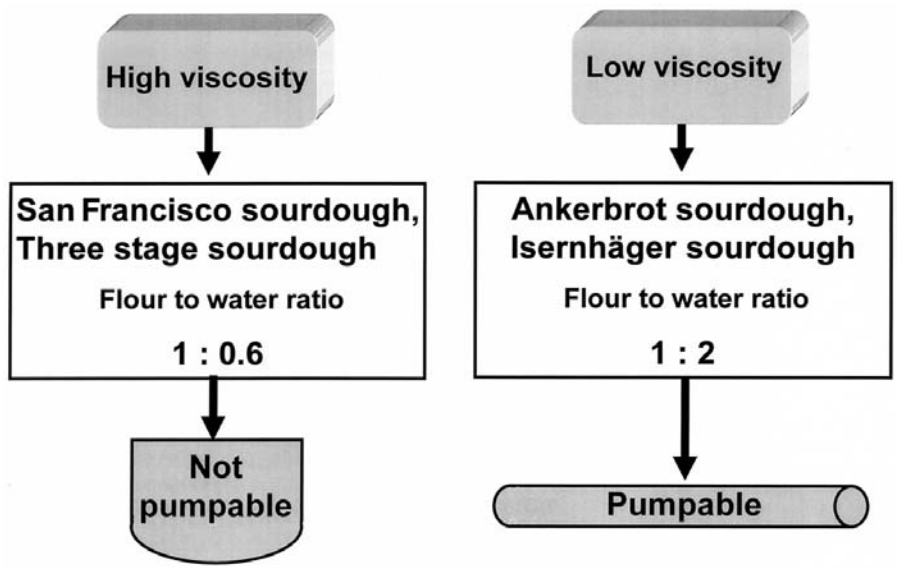
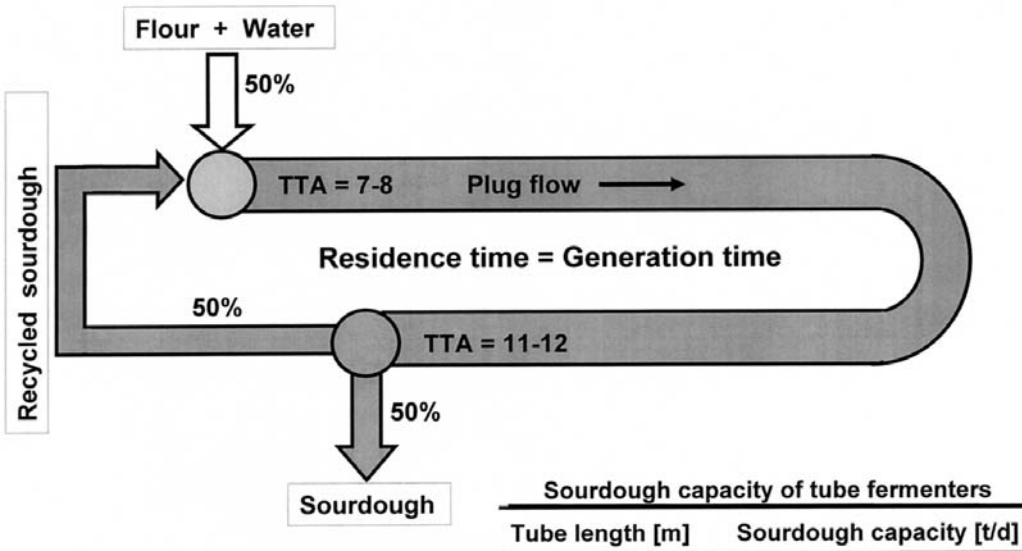


Figure 5 Distinction of sourdoughs by their viscous properties.

B. Principles of Continuous Sourdough Fermentation

The principle of the most advanced continuous sourdough fermentation consists of pumping a dough through a fermenter in plug-flow pattern (Fig. 6). This enables a gradient to be established for the changes that occur in the sourdough between fermenter inlet and outlet, the magnitude of which results from the residence time of the sourdough in the fermenter. Continuous sourdough fermentation requires the gradient to be constant with respect to the number of metabolic active cells. The bacteria count must be high enough to permit a degree of acid formation adequate for the intended purpose of the sourdough (38).

During sourdough fermentation, the gradient is established by feeding a portion of the fermenting dough taken from the outlet back into the inlet of the fermenter. This dough serves as an inoculum that is mixed with fresh sourdough to maintain a constant mass flow. The condition for upholding continuous fermentation requires that with a constant bacteria count at the outlet of the fermenter, the gradient or the ratio between the respective bacteria counts at the inlet and outlet of the fermenter remains constant. The residence time of the sourdough in the fermenter depends on the generation time resulting from the fermentation conditions. Consequently, the generation time determines the productivity of the fermenter, which is defined as the mass of sourdough that can be taken from the fermenter within a unit of time. Assuming a constant generation time, the maximum productivity of the fermenter is equal to half the mass flow through it. The mass flow of sourdough that can be withdrawn from the fermenter decreases as the ratio of the inoculum to the total amount of dough diminishes.



Sourdough capacity of tube fermenters

Tube length [m]	Sourdough capacity [t/d]
50	5.5
100	11.0

Tube diameter = 200mm, average sourdough density along the tube length = 0.9kg/L

Figure 6 Principle of the tube fermenter.

Sourdough requires high acid formation by lactic acid bacteria. This is possible only if there is a high bacteria count. When the bacteria count is high, acid formation follows the development of the bacteria count (38,39). For the operation of the fermenter it is of considerable practical advantage that proliferation is self-regulating in its transition phase. This can be demonstrated theoretically on the basis of a logistical proliferation model (40).

In this model (Fig. 7), the theoretical development of the proliferation of a lactic acid bacteria population is presented for different initial bacteria counts in the area of the transition phase, assuming constant values for the generation time and the maximum attainable count (39). For example, a relatively high initial population can no longer double within a defined period. With an initial inoculum to total dough ratio of 1:1, the bacteria count is halved for each new sourdough set. The new set has a smaller initial population than its predecessor. Due to the smaller initial population, the microorganisms multiply more rapidly during the fermentation time so that the population doubles. As long as the residence time is the same as the shortest generation time in the exponential proliferation phase, the culture cannot be washed out of the sourdough in continuous operation of the fermenter.

It was this invention that provided the crucial breakthrough for all subsequent methods of continuous sourdough fermentation based on the same principle. The first system to

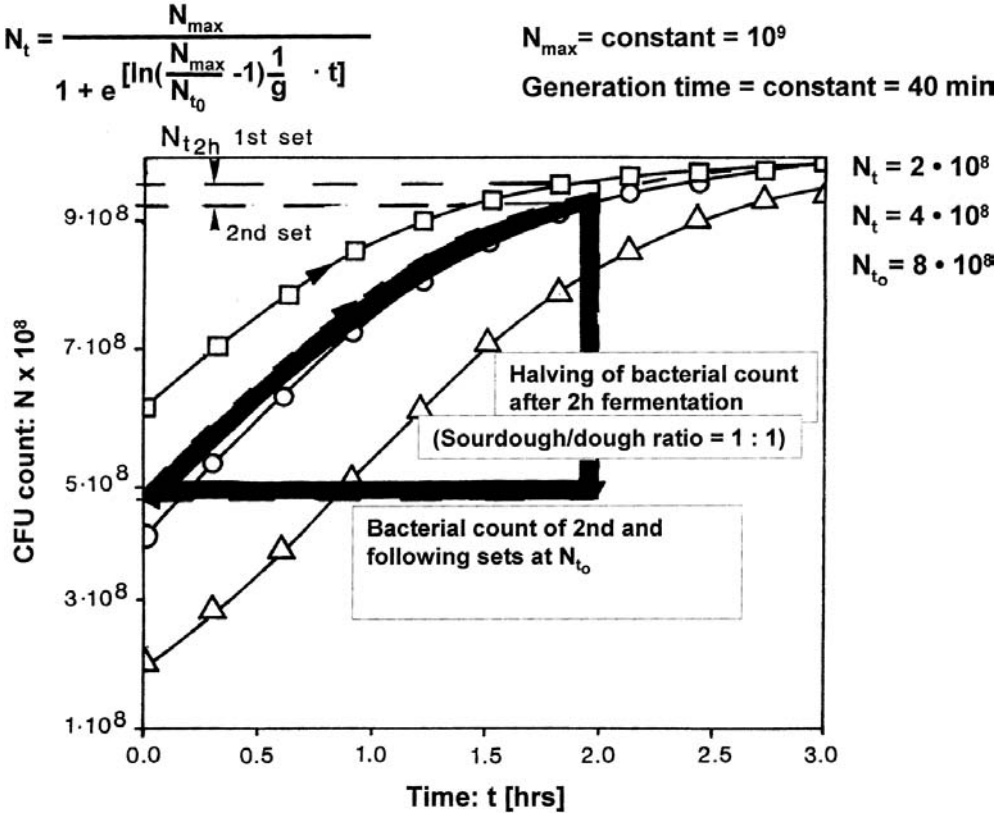


Figure 7 Theoretical consideration of the influence of the initial bacteria count on the proliferation of the lactic acid bacteria.

be based on it was the Ankerbrot-Reimelt system (ARS) (41,42) which was developed to produce large quantities (20 tons/day) of sourdough. The system comprises a fermentation tube and fermentation tank. The principal function of the fermentation tank is to multiply the sourdough.

The ARS has two specific advantages. First, sourdough can be cooled in the fermentation tube to bridge interruptions in bread production. Second, a short period of time (less than 4 hr) is required to prepare a large mass flow of sourdough for breadmaking. The ARS is well suited to operations with a large sourdough requirement but with only small variations in production and a small assortment of bread.

However, these are also disadvantages. The system cannot handle variations in daily and weekly production rhythms, and a variety of sourdoughs cannot be prepared simultaneously. Therefore, a new course had to be followed to meet these requirements, which led to the development of the Paech-TUB-Reimelt system (PTRS). PTRS is based on the principle of combining continuous and batch-wise sourdough fermentation to vary production in response to hourly, daily, or weekly production rhythms (43).

The system consists essentially of two fermenters, between which an insulated tank is located (Fig. 8). A heat exchanger is situated downstream of the first fermenter (43). This fermenter is a narrow, cylindrical, insulated tank through which sourdough flows continuously. The tank downstream of this fermenter is also cylindrical but has a considerably larger diameter. This tank functions as a storage vessel that is filled from above and emptied from below. The tank is fitted with a stirrer to facilitate the withdrawal of the cooled dough, which does not flow as easily as freshly fermented dough owing to its relatively higher density and higher viscosity. The second fermenter is constructed as a segmented tank, the segments of which are also filled from above and emptied from below.

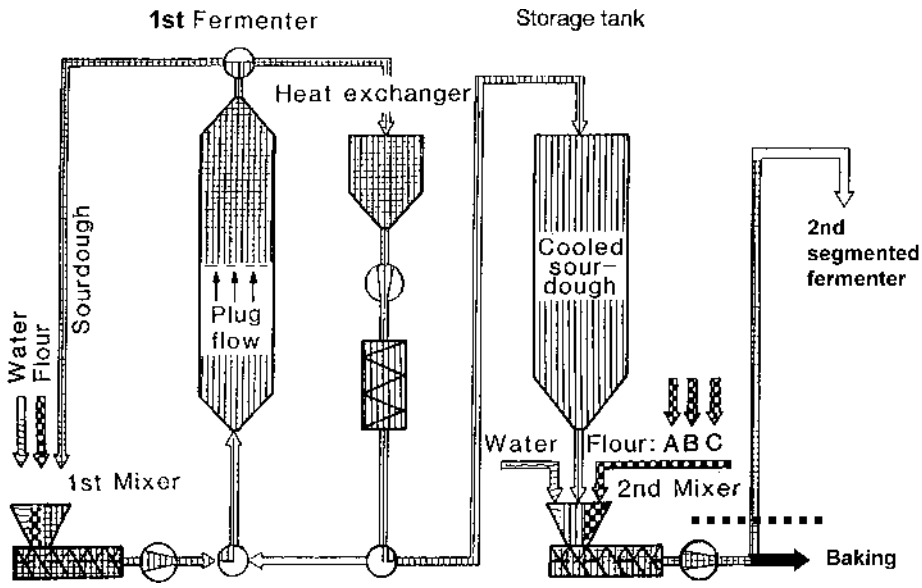


Figure 8 Schematic diagram of the most advanced continuous sourdough fermenter (Paech-TUB-Reimelt system).

The advantage of the PTRS is that the first stage can be operated continuously over a long period of time. The sourdough in the storage tank can be used to meet the different requirements for the supply of sourdough. Different quantities can be taken quickly from the storage tank to prepare and propagate fresh sourdoughs. In the PTRS system, fermentation in the second stage is intermittent because the quantities of sourdough required for each type of dough are too small for continuous production to be profitable.

The system can also be operated with just the first continuous stage in which the continuously fermented sourdough, which is cooled as required, does not necessarily need to be propagated but instead can be used for making bread immediately. The advantages of propagation to produce a variety of sourdoughs are lost in this case. However, sourdoughs can be supplied from the storage tank in widely varying quantities at virtually any time. Just as so-called "no-time" wheat flour doughs can be transferred directly from the mixer to the divider, these ready-to-use sourdoughs may be referred to as "no-time" sourdoughs. This is the second key breakthrough for the present-day preparation and use of sourdough because it ensures highly flexible bread production in terms of bread varieties and quantities per time unit.

The sole use of the first continuous stage has now become the established way of preparing sourdoughs. Although the advantage of being able to flexibly produce different types of sourdough is forfeited, many plant bakeries in practice mostly require only a single type of sourdough in day-to-day production, although it may be combined with other preliminary doughs as necessary to maintain a wide assortment of bread.

As regards the design of the plant, a fermentation tube has been substituted for the fermentation tank. This became necessary as the tube enables sourdough fermentation to be integrated into the bread-making process more readily than the tank. This is explained by the fact that the small cross-section of the fermentation tube enables the dough in the tube to be cooled rapidly and effectively by means of a cooling jacket in order to inactivate the metabolism of the microorganisms. The poor thermal conductivity of the frothy sourdough aside, the sourdough cannot be cooled as effectively in the tank owing to the latter's diameter, which is four times larger than that of the tube. Tanks must therefore be emptied completely and cleaned whenever operation is interrupted, but the tube can remain filled.

Although cooling the dough results in an increase in its density, as shown by the drop in the filling height over the cross-section of the tube, there are no disadvantages for restarting operation of the tube fermenter. The increase in the density of the dough due to cooling enables heat to be transferred within a short period of time when the operating temperature at which fermentation takes place has been reached so that the metabolic activity of the microorganisms is quickly set in motion. This results in a renewed volumetric expansion of the sourdough and in the cross-section of the tube being filled. In addition, the tube cross-section is rapidly refilled by sourdough being pumped into it, resulting in the required plug-flow. The tube fermenter is therefore fully functional as soon as the sourdough has been heated to its operating temperature.

This type of sourdough plant is suitable for fermenting both large (>10 tons/day) and small (<1 ton/day) quantities of sourdough. It has hitherto been designed mainly to achieve a high level of performance of the continuously operating tube fermenter owing to the relatively high level of investment required to integrate it into the bread-making process. Above all, the investment is worthwhile, considering the immediate availability of sourdough whatever the quantity required, the increased reliability of production, and the new possible realization of manpower. The resultant economic advantages of continuous sourdough production for bread-making are so great that they outweigh the cost of producing around 25% more sourdough than required for discontinuous three-stage

sourdough production in troughs for the same quantity, of bread. As has already been explained, the larger quantity of sourdough is the result of the differences in the acidity of the two types of sourdough.

These advantages aside, one of the disadvantages of this type of continuous sourdough fermentation is the limitation of the final acidity (total, titratable acidity = TTA) in the cooled sourdough to a maximum of around 12 due to the fermentation time being linked to the generation time of the microorganisms. Thus, the leavening potential of the milled product used, which depends first and foremost on the latter's phytate content, is nowhere near exhausted. Owing to the low acidity of this type of sourdough, the proportion of flour that needs to be leavened when making bread dough is around 25% greater than that used to ferment a three-stage sourdough when compared with the same level of acidity in the final dough.

Another difference between the two types of dough is that the pH decreases more slowly during the third stage of the batch-type fermentation of the three-stage sourdough than during continuous sourdough production. This is due to the higher content of lactic and acetic acids (around 0.33%) in the inoculated continuously proliferated sourdough (42) as compared to the lower content of these acids in the inoculated third stage (around 0.16%) of the three–stage sourdough (23). In case of the continuously proliferated sourdough, the large quantity of inoculum (50% of the total sourdough) leads to the development of a microorganism biotope in the tube fermenter. The biotope remains stable for a long period of time (years) and is thus similar to a three-stage sourdough, which is started with a set-aside portion of the previous third stage. Consequently, the sourdough does not need to be reinoculated with a sourdough starter. In this respect, it is similar to three-stage sourdoughs, although it differs from these in ripening times, which may affect the aroma profile of the end products.

C. Batch-Type Production of "No-Time" Rye Sourdoughs

All classical methods of sourdough production were developed on the basis of a daily rhythm of bread production. As it is not possible to exhaust the buffering capacity of common rye flours within a 24-hr period in any type of sourdough production, it was never considered that it might be possible to reduce the proportions of sourdough required by bringing about the highest possible degree of acidity in the sourdough. Such thoughts are also inconsistent with the ripening regimens for rye sourdoughs, such as the regimen that has been developed to perfection for three-stage sourdough, for example, which focuses on the production of precursors of flavor components.

Figure 9 shows a three-stage sourdough fermentation method that was used in a bread factory in Berlin. It shows that it took more than 20 hr to ferment the sourdough needed for bread dough production. Bulk fermentation took place during this time, and the dough was proliferated in three stages. The batchwise preparation of sourdough, which requires many vessels in a large factory, is time-consuming and difficult to automate and integrate into computer-integrated manufacture.

It was not until mechanization of production processes and rationalization of manpower began to displace this type of sourdough production, particularly in small bakeries, that the possibility of raising the acidity of sourdough was considered. The invention of Isernhäger rye sourdough provides an astoundingly simple solution, which has become an important breakthrough in sourdough production (Fig. 10). The Isernhäger process focuses on producing a high acidity (TTA = 28–32) in liquid sourdoughs (dough yield: 200–250%). The idea behind the invention is to produce a high level of acidity by exploiting the buffering

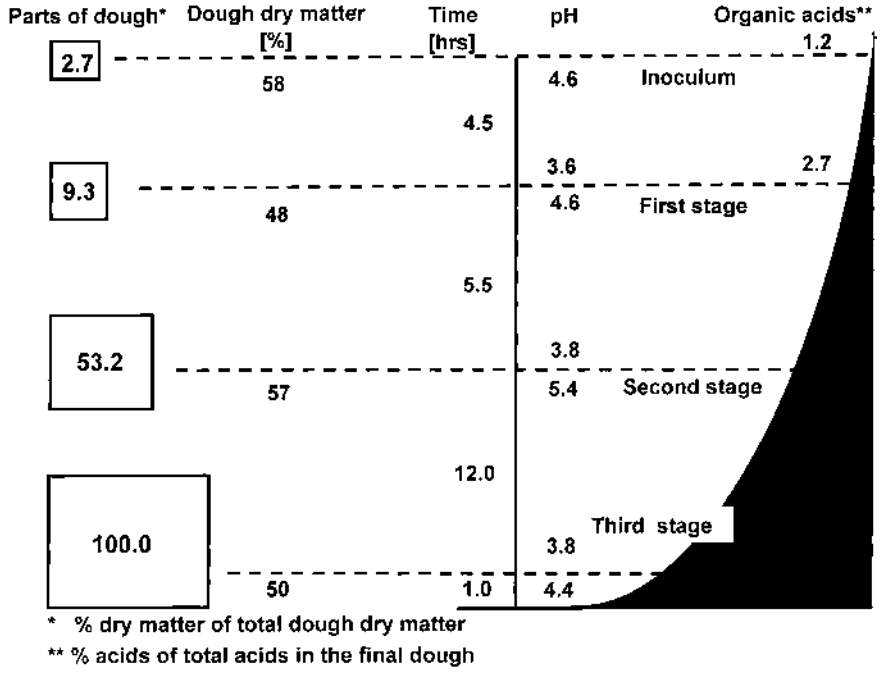


Figure 9 Course of a three-stage sourdough fermentation.

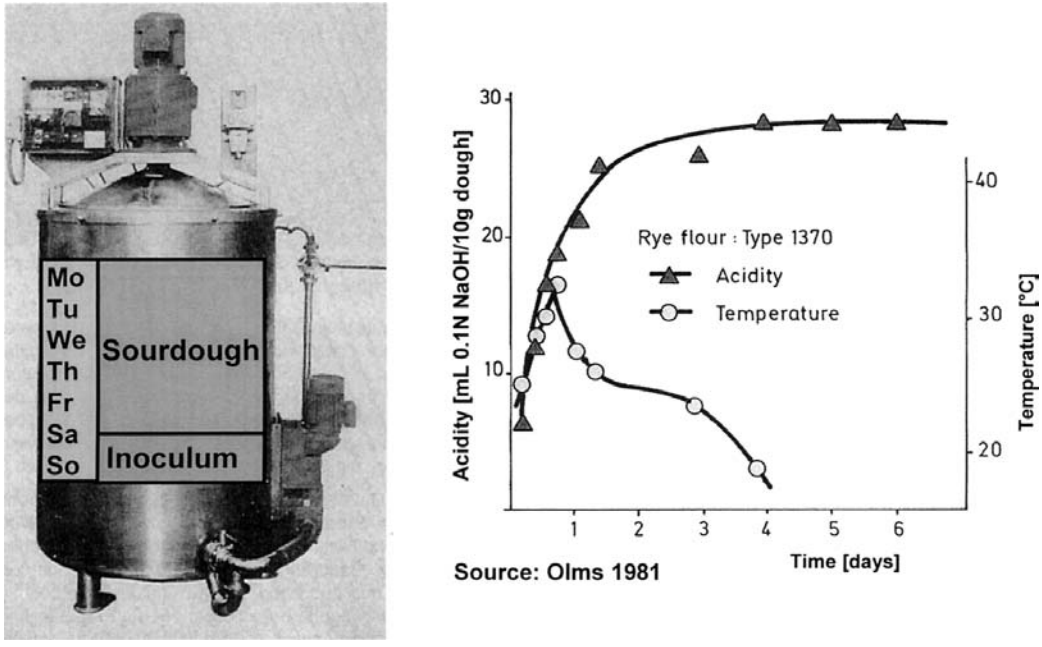


Figure 10 Isernhäger sourdough fermentation.

capacity of the substrate. Isernhäger rye sourdoughs are produced in a fermentation tank fitted with a stirrer. The fermentation tank is not cooled. A siphon through which the sourdough is pumped out is fitted on the base of the fermentation tank, which can have a capacity of up to 2 m^3 (without a cooler). The height of the siphon outlet determines the quantity of sourdough remaining in the tank, which serves as an inoculum when the fermentation tank is refilled each week. The maximum capacity of the fermentation tank and the final density of the sourdough determine the maximum quantity of sourdough available each week. The fermentation tank is designed in such a way that its surface releases the heat generated by fermentation into the environment (44). The sourdough is also stirred continuously to aid the cooling process. At the same time, the volumetric expansion of the sourdough that accompanies the formation of CO_2 is limited. It takes around 48 hr for fermentation to be completed. The sourdough then has such a low pH (3.6–3.8) that the metabolic activity of the microorganisms is largely reduced to the basal metabolic rate (45). Sourdough in this form is microbiologically stable. It can therefore be stored for weekly breadmaking rhythms as its acidity remains virtually constant.

We were able to demonstrate that Isernhäger sourdough prepared in accordance with the process specifications over a relatively long period of time (2 weeks) is not only microbiologically active but is also stable and will keep throughout its intended storage period (46). For the purposes of verification, the TTA and the lactic acid bacteria and yeast counts (colony-forming units [CFU]) were determined during fermentation. The metabolism of the microorganisms present in the sourdough during prolonged storage was calculated from the measured values (Eq. 1).

$$\beta_E = \frac{1}{CFU} * \frac{\mathrm{dTTA}}{\mathrm{dt}} \qquad \text{Eq. (1)}$$

In Eq. 1, β_E is the coefficient of acid formation irrespective of the growth of lactic acid bacteria, CFU is the lactic acid bacteria count per gram of sourdough, and dTTA/dt is the increase in the TTA with time (TTA rate). The TTA rate was calculated from the measured values using a linear regression equation (Eq. 2) in which b is a constant.

$$\mathrm{TTA} = \mathrm{b} + \frac{\mathrm{dTTA}}{\mathrm{dt}} * \mathrm{t} \qquad \text{Eq. (2)}$$

Figure 11 shows that the CFU of the lactic acid bacteria and yeasts decreased only slightly during the storage period. The coefficient β_E of the metabolism during prolonged storage ranged from 3.53 to 6.20 $\times$ 10^{-12} (TTA $\times$ hr^{-1} $\times$ CFU^{-1}) for the sourdoughs concerned. The results demonstrate that the lactic acid bacteria in the sourdough are active during prolonged storage, that the sourdough will keep owing to the high TTA and low pH, and that the sourdough is stable by virtue of the low acid formation accompanying its low metabolism activity during prolonged storage. The lactic acid production rate during prolonged storage was virtually constant as shown by the upward slope of the curves (Fig. 12). However the TTA rate, which corresponds to lactic acid production, was different for each of the sourdoughs under investigation (2.50–3.23 $\times$ 10^{-2} (TTA hr^{-1}). From this it follows that various species of lactic acid bacteria also exhibit different levels of metabolic activity during the phase of prolonged storage on an otherwise constant substrate.

Storage is facilitated in particular by its high acidity, which is twice as high as that of the ready-to-use three-stage sourdough. The quantity of sourdough needed to make sourdough bread with Isernhäger sourdough is therefore half that required when using three-stage sourdough.

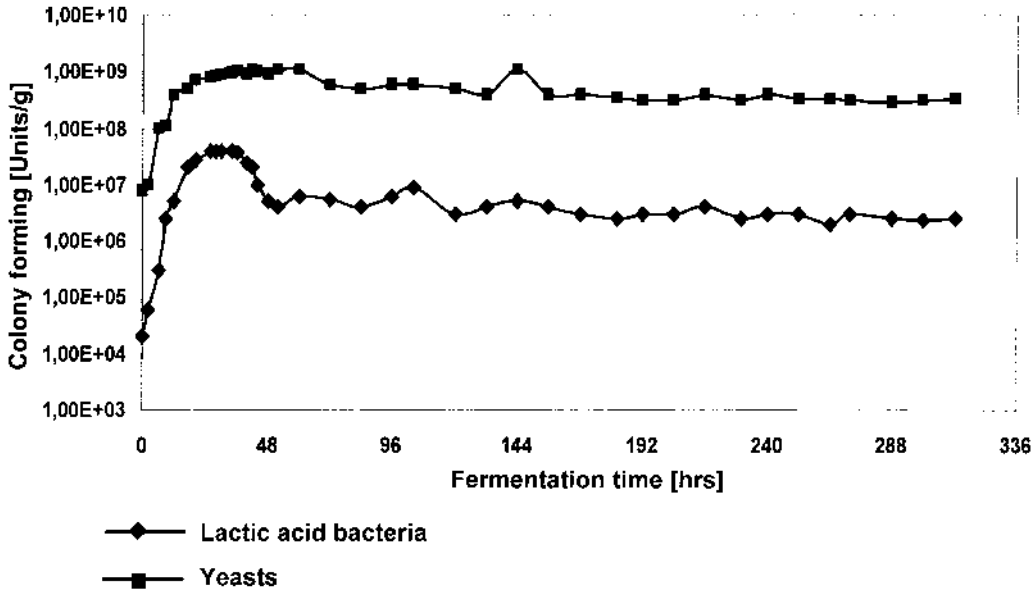


Figure 11 Development of the microorganism count.

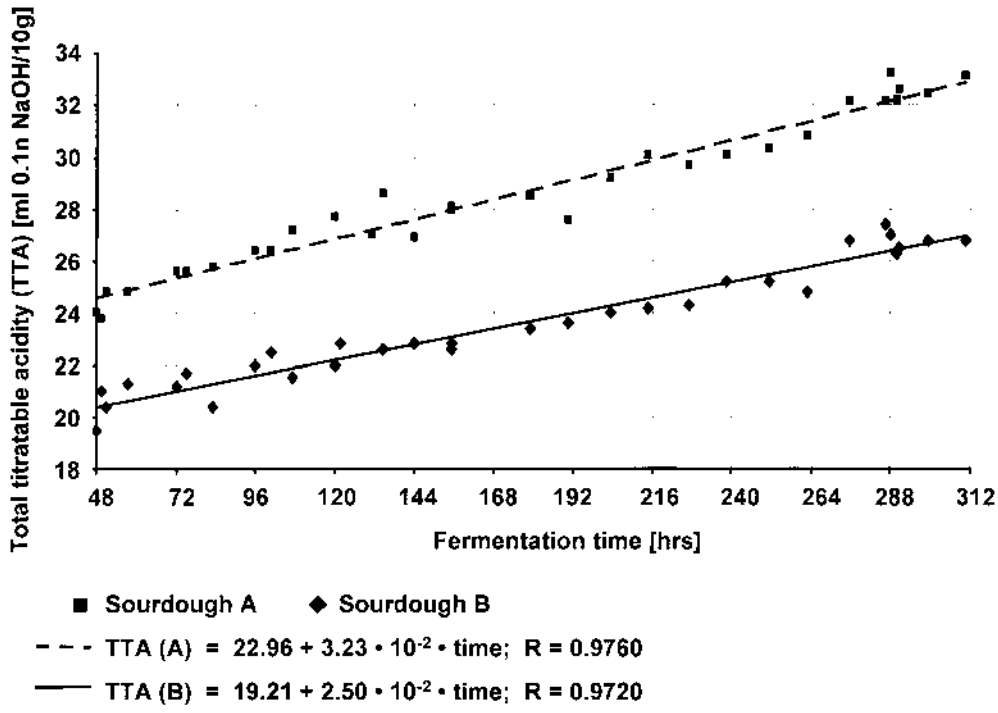


Figure 12 Development of the total titratable acidity during constant metabolism activity.

The quantity of sourdough remaining in the fermentation tank and used as an inoculum for the weekly production of Isernhäger rye sourdough ranges from 7% to a maximum of 30% of the total amount of sourdough produced in a single batch (45). The quantity remaining in the fermentation tank can be used as an inoculum several times over. A special starter (Isernhäger GmbH & Co.) is substituted for the inoculum in the fermentation tank whenever the metabolic activity diminishes to such an extent that the final acidity cannot be achieved rapidly enough. This happens when the vitality of the microorganisms decreases and the spectrum of microorganism flora changes to the detriment of the dominant species of microorganism. The starter is proliferated on milled products and contains a mixed culture in which lactic acid bacteria species dominate. The dominant metabolic activity of these starter species results in bakery products with a mild flavor. In addition to this species, the lactic acid bacteria species present in the flour also proliferate in the starter (45).

Owing to the rapid formation of acid and the low pH after the final acidity has been reached, the metabolic activity of the microorganisms is reduced to the basal metabolic rate and the activity of the endogenous enzymes in the flour is limited to such an extent that formation of precursors of flavor components is virtually halted. Furthermore, as only half as much Isernhäger sourdough as three-stage sourdough is used in the total bread dough and the microorganisms in the Isernhäger sourdough do not exhibit any significant metabolic activity during the dough and loaf ripening times, Isernhäger rye sourdough contributes less to the formation of the flavor profile of bakery products than the three-stage rye sourdough.

This disadvantage has been overcome by another invention that also constitutes a breakthrough in sourdough production. The invention consists of including bread (either whole loaves or sliced bread) in the fermentation substrate and utilizing the stirrer designed with cutting blades together with a basket-like feeding device in the fermentation tank to cut up the bread. This results in a pulpy sourdough containing, in particular, the flavor substances present in the bread crust. In addition, this type of sourdough is enriched with the gelatinized starch present in the crumb. It can therefore also perform the same function as preliminary doughs containing gelatinized starch.

In addition to this, including bread returns in the sourdough recipe is particularly hygienic and economical. Under the conditions specific to the manufacturing process, the bread is broken up and converted into a homogeneous mass that can be used to make well-leavened bread with an aromatic flavor that keeps its freshness well. These advantages are obtained in particular when there is a high concentration of bread (50%) in the sourdough (45).

D. Modern Small-Scale Wheat Sourdough Production

While it is essential to use rye sourdoughs in order to achieve the required baking performance of bakery products containing rye flour, this does not apply to the use of wheat sourdough for bakery products made of wheat milled products. Wheat sourdough is included in recipes primarily to increase the flavor of the bakery products (panettone) or to emphasize a particular flavor profile (Sanfrancisco sourdough bread). Sourdoughs with a very low dough yield (<150%) are used for this purpose. The advantage of this type of production is the long fermentation time and low level of acid formation (TTA = 12) that are essential for the development of the typical characteristics of this type of sourdough, with the precursors of flavor components governing the formation of the quality characteristics. The use of yeast leavens with long fermentation times, such as sponges, has the same objective. Although not brought about intentionally, sourdough bacteria proliferation and

acid formation takes place in sponges, the extent thereof depending on the fermentation time and conditions.

Liquid sponges for use in dough fermentation have the considerable advantage of being pumpable, which sets them apart from the firm, nonpumpable wheat sourdoughs. This major criterion for the mechanization of sourdough production has recently led to the development of wheat sourdough plants. The designers of such plants have applied findings resulting from the use of rye sourdough plants and pumpable sponges. Gluten formation in wheat flour doughs with dough yields greater than 200% presented a considerable problem for the development of such plants, which has only been solved empirically so far.

The aim was to mix flour with water in such a way as to obtain a dough in which the gluten develops only to the extent that, together with the starch, it forms a homogeneous and stable dispersion that is also maintained on lactic acid formation. When dispersions of this kind are subjected to an excessive input of mechanical energy, the elastic properties of the gluten can develop to such an extent that the gluten agglomerates and releases starch granules. The latter separate out and, as a result, the dispersion cannot be maintained and loses its pumpability. The risk of changes in the rheological properties of the dispersions is the main obstacle to the construction of wheat sourdough plants. The successful design for example by the Isernhäger Landkost company of fermentation plants in which flour-in-water dispersions remain stable throughout the fermentation time in each stage of sourdough production can be regarded as a major breakthrough in wheat sourdough production.

The wheat sourdough plant comprises a mixing vessel (volume: 0.5 m^3) and two temperature-controlled fermentation tanks (volume: 2 m^3) operated in tandem. The mixing vessel is fitted with a specially designed propeller mixer and is used to fill the fermentation tanks during batch-wise operation. The inoculum—in this case a wheat sourdough starter—is added to the first batch. The quantity of inoculum depends on the quantity of sourdough in the fermenter, which in turn depends on the quantity of sourdough required for producing baked goods on the following day.

Operating the fermenters on alternate days requires foresighted planning in the same way that production of three-stage rye sourdough does, for example. Once the operating schedule has been laid down, it cannot be altered. Such operating schedules for the sourdough plant therefore rule out any flexible response to sudden variations in the sourdough requirement. However, it is possible to store a certain amount of sourdough as the filling volume of the fermentation tanks is exploited to the full and the sourdough can also be cooled to 10°C. Unfortunately, the maximum storage period is 48 hr for both mechanical and microbiological reasons: gluten agglomeration on the one hand and the fact that metabolic activity is not completely suspended at 10°C on the other hand (43).

Each fermentation tank is fitted with a wall-scraping anchor stirrer. The fermenting dough is mixed at a low energy input, mixing being halted completely at intervals. The sole purpose of mixing is to ensure that the fermenting sourdough is distributed evenly, has the same temperature throughout and that its volumetric expansion is limited. The sourdough in the fermentation tanks can be heated or cooled by means of the temperature-controlled jacket. Heating serves mainly to reach and maintain a constant operating temperature, and cooling reduces the metabolic activity so that the sourdough can be stored. The wheat sourdough in the fermentation tanks takes at least 16 hr to ripen.

This type of wheat sourdough is a "no-time" sourdough intended for storage within a daily production rhythm. The basic principle for its production originated in the Isernhäger tank fermentation for sourdough. On the base of its two fermentation tanks, the plant can be operated semicontinuously to produce sourdough for bread-making. Sourdough produc-

tion is restricted owing to the technical limits placed on the size of the fermentation tanks fitted with stirrers.

Such limits do not present any problems as long as bakery products contain only a small proportion of sourdough and the bakeries involved have small capacities for producing individual types of baked goods. The proportion of this type of sourdough added to baguettes, for example, is normally less than 10% in relation to the quantity of flour used. From this it also follows that there are as yet no wheat sourdough plants for large bakeries making products in which a large proportion of wheat sourdough is used, such as Sanfrancisco sourdough bread.

E. Outlook for Modern Large-Scale Wheat Sourdough Production

The concept for a plant capable of manufacturing liquid sponges developed by the Reimelt company constitutes a new approach to the development of large wheat sourdough plants. Reimelt has developed a plant for the fermentation of liquid sponge, which constitutes a technical breakthrough in sponge production. The capacity of the liquid sponge plant is 2000 kg/hr at a fermentation time of 4 hr. The plant is operated with wheat flours with a high gluten content (wet gluten >30%) and pronounced viscoelastic properties.

The plant consists essentially of a system of mixing flour, water, and yeast, two fermentation tanks operated in tandem, a plate cooler, and an insulated storage tank fitted with a stirrer. There are no stirrers in the fermentation tanks, which are filled from above and emptied from below. The principle of first-in first-out as applied in fermentation tubes has thus been adopted here, too. The same principle was also applied to the continuous Reimelt rye sourdough fermentation tank, except that the tank is filled in a continuous flow from its base to its top. This mode of operation was not feasible for liquid sponge fermenters designed without a stirrer as the fermentation tanks act as ripening tanks in a batchwise mode of operation.

The ripened liquid sponge is pumped through the outlet in the base of the tank and over the plate heat exchanger into the storage tank. Owing to the stable gluten network, which is interspersed with gas and determined by the flour characteristics, no mixing of the individual layers of dough occurs over the height of the fermentation tank. Therefore, the tank is emptied in a plug flow pattern. In respect of the flour characteristics, the critical density of the liquid sponge must not exceed a value of 0.65 g/cm^3. The critical density of the fermenting sponge can only be limited by means of the quantity and dough-raising power of the yeast used, as it is not possible to cool or stir the fermentation tank.

Such sponges develop an acidity of around 5 mL TTA mainly owing to the formation of acetate by the yeasts. The acidity could only be increased within the fermentation time of 4 hr if an inoculum of lactic acid bacteria were added to the sponge. However, this possibility is still in an experimental stage. It is for this reason that the problem of how to design large-scale plants for semicontinuous or continuous wheat sourdough cannot yet be considered to have been totally solved.

In the context of such developments in processing technology, it should be noted here that, in accordance with the definition laid down by Olms (47) (Fig. 13), an acidity of 7 mL TTA constitutes the boundary between the two types of preliminary dough. The rheological and microbiological requirements as well as the requirements for processing technology need to be fulfilled in order to resolve the problem deriving from that definition. The microbiological requirements consist first and foremost in selecting microorganisms (lactic acid bacteria and yeasts) that form a large number of precursors of flavor components but few organic acids (lactic acid and acetic acid) and carbon dioxide in a short fermentation

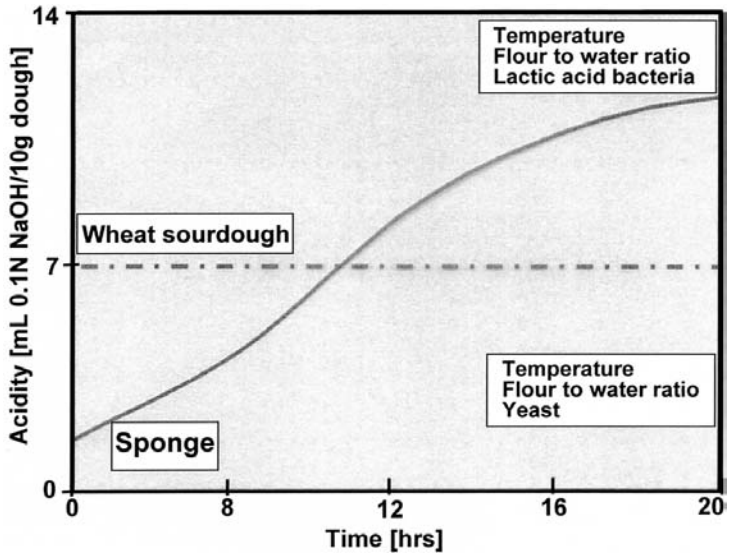


Figure 13 Distinction between sponge and wheat sourdough.

time. The formation of lactic acid is particularly important as it can destabilize the gluten network.

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Packaging, Quality Control, and Sanitation of Bakery Products

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I. INTRODUCTION

Since the dawn of civilization, bread has been a central part of the human diet. Then, and until recent times, most bread was eaten fresh because the raw materials were ready available. In modern society, industrialized bakeries have overtaken domestic bread-making and outcompeted many small bakers; thus, most bread are bought in supermarkets and are expected to stay fresh for several days, which of course has put extra demands on bread factories for longer products shelf life. The preferred way to handle this problem has been to add large amounts of chemical preservatives, such as propionate and sorbate, to bakery products. However, public demand for more natural foods, without synthetic preservatives has increased in strength since the early 1970s and has forced bread factories to minimize the use of preservatives.

This chapter will focus on spoilage problems related to bread and bakery products and ways to extend shelf life through sanitation and improved hygiene in the bakery plant, and preservation with traditional preservatives, the use of sour dough, or by natural antimicrobial substances from plants—either alone or in combination with modified atmosphere packaging and active packaging.

II. SPOILAGE OF BAKERY PRODUCTS

The spoilage of bakery products is mainly caused by molds and yeasts and only occasionally by bacteria. This is due to a reduced, water activity and for some products, also a reduced pH. The spoilage fungi are fastidious organisms, and a shelf life of only 2–3 days may be expected for products like unpreserved wheat bread, especially if the hygiene in the factory is not sufficiently high. Besides the repelling sight of visible growth, fungi are responsible for off-flavor formation and the production of mycotoxins and allergenic compounds. These compounds may be formed even before growth is visible.

A. Bacterial Spoilage

In some cases, bacteria may cause a serious spoilage problem in bakery products. In white and wholemeal wheat bread, the heat-resistant endospore-forming *Bacillus subtilis* is known to cause ropiness (1–3), especially if the bread is produced without preservatives or sour-dough (4). Other *Bacillus* species such as *B. licheniformis* and to some extent also *B. cereus*, may survive the baking process despite their lower heat resistance, but they will not cause ropiness (4). *B. cereus* is capable of causing food-borne infections and intoxications, but *B. subtilis* and *B. licheniformis* have also been implicated in food poisoning (5). Another and more serious spoilage organism is the anaerobic bacterium *Clostridium botulinum* which may grow and produce toxins in high-moisture bakery products like English-style crumpets (pH 6.5 and a_w 0.99) when packaged in modified atmosphere (6). Crumpets were toxic after 4 and 6 days respectively, when inoculated with 5×10^2 bacterial spores per gram after or before baking. The packaging conditions did not influence toxin production. Consequently, high-moisture bakery products contaminated with *C. botulinum* spores could pose a public health hazard when stored at room temperature. Bakery products with unbaked cream fillings are sensitive products and the production and handling of these products in small bakeries with insufficient hygiene have been responsible for many cases of food poisoning (7). These are, however, all related to the cream filling and will thus not be discussed further.

B. Fungal Spoilage

Most vegetative cells and mold spores are expected to be killed by the high temperature in the baking process, thus after-contamination is the source of spoilage problems. Some heat-resistant molds can survive the bread-making process in some cases, but these organisms have not been reported as spoilage organisms on bread. Contaminants of wheat bread are mostly *Penicillium* species (90–100%) and, to a lesser degree, *Aspergillus* and *Clodosporium* species (3). The most important mold species associated with wheat bread are *P. commune*, *P. crustosum*, *P. brevicompactum*, *P. chrysogenum*, *P. roqueforti*, *A. versicolor*, and *A. sydowii* (8). On rye bread, *P. roqueforti* is the major contaminant (9–11). In a 4-year investigation of rye bread in Denmark, *P. roqueforti* (27%), *P. corylophilum* (20%), and *Eurotium* sp. (15%) (*E. repens*, *E. rubrum*) were identified as the most important species. Looking at all these isolates of the important spoilage organism, *P. roqueforti*, it was clear that they formed three distinct groups. These were further investigated by chemical and molecular methods and shown to be three distinct but closely related species: *P. roqueforti*, *P. paneum* and *P. carneum* (12). Of these *P. roqueforti* and *P. paneum* dominate on bread, *P. carneum* on meat.

The yeasts known as chalk mold are also common on bread but mostly on sliced bread: in one survey, they accounted for 5–30% of the spoilage organisms (3). On rye bread, the chalk molds *Endomyces fibuliger* and *Hyphopichia burtonii* are the dominant yeast species (9,11).

Rychlik and Schieberle (13) studied mycotoxin production by a less common bread spoilage funga, *P. expansum*, on wheat bread. They found high concentration of the mycotoxin patulin at the surface and were able to detect it down to 4 cm from the infected spot. Other toxins may be able to diffuse even longer in bread, as demonstrated for aflatoxin that may be produced by *A. flavus* on bread (14). The production and diffusion rate in bread, are however, not know for the toxins more likely to be found in bread: roquefortin C from *P. roqueforti* and *P. chrysogenum*; PR-toxin from *P. roqueforti*; cyclopiazonic acid from *P. commune*; patulin and penitrem A from *P. carneum*; patulin from *P. carneum*; and sterigmatocystin and nidulotoxin from *A. versicolor* (15). Despite that, it is still evident that it is

of great importance to hinder mold growth on bread and to discard bread when it becomes mouldy and thus avoid the use of infected bread for human consumption or cattle feed.

Cakes typically have a much lower water activity than breads (a_w 0.70–0.85 as compared to 0.92–0.97) and a much higher pH (6.0–8.0 as compared to 4.4–4.8 for bread started with sourdough and around 5.7 for wheat bread started with yeast alone) (13,16). This will of course, favor another group of fungi. Thus, the fungi associated with cakes, also termed the associated funga of cakes, will typically consist of *Wallemia sebi*, *Eurotium* spp., and *Aspergillus* spp. such as *A. flavus* and *A. niger* (5,17,18). The osmophilic yeast *Zygosaccharomyces rouxii* has also been isolated from spoiled cake with high sugar content. A predictive model for growth of this organism in bakery products has been developed by Membré and coworkers (19). They used a mixture of 30% glucose and 70% sucrose to simulate the mixtures commonly used in bakery goods and found that growth rate decreased linearly with increasing sugar content up to 950 g/L, corresponding to a_w 0.788. The optimal pH for growth was in the range 3.5–5 (19).

III. QUALITY CONTROL AND SANITATION OF BAKERY PRODUCTS

In a 4-year study of Danish rye bread factories, Lund and coworkers (11) discovered that the associated funga varied through the season. They argued that air sampling and swab tests combined with the use of selective media and identification of the molds and yeast to species level in many cases can show the source of contamination and thus provide a way to solve the problem. These tests showed that the slicing machines were a very critical point for contamination with chalk molds (*Endomyces fibuliger* and *Hyphopichia burtonii*). Cleaning and disinfection of the emulsions of cutting oil in the machine immediately reduced the frequency of chalk molds on bread. *Eurotium* species were found in high numbers during periods where ethanol (70%) was used as the primary disinfection agent, as the ascospores of *Eurotium* are highly resistant to alcohols (20,21). A change to chlorine dioxide, hypochlorite or—even better—the quaternary ammonium compound benzalkonium chloride would solve that problem (22). The source of *Eurotium* was assumed to be wooden pallets in the bread factory, as their funga was dominated by *Eurotium* species (11).

Benzalkonium chloride was efficient against most fungi except *P. roqueforti* and *P. carneum*. These fungi also showed considerable resistance towards alcohols, whereas 3% hypochlorite efficiently eliminated them. Most yeasts are effectively controlled by the quaternary ammonium compounds, 70% ethanol, 70% isopropanol, and 30% hypochlorite (21).

Resistance of molds and yeasts strongly depends on strain and species of the isolate and active component of the disinfectant. Different isolates of one species may show different response to the same disinfectant, resulting in an effective kill in one case and almost no effect in others. To obtain proper manufacturing hygiene, it is, therefore, important to know the resistance of the fungi that are associated with the process and the product and from this knowledge select proper disinfectants. The use of one disinfectant is usually not enough. It is necessary to make a disinfection plan whereby at least two different disinfectants containing different active components are used in rotation during the week to keep the contamination of molds and yeasts in control and to avoid selection of super-resistant isolates (21). However these initiatives should not stand alone but rather be a part of a Hazard Analysis and Critical Control Point (HACCP) system (18) as set out by Codex alimentarius in their Recommended International Codes of Practice, General Principles of Food Hygiene (22). The controls described in this document are internationally recognized as essential to ensure safe and healthy food.

IV. PRESERVATION METHODS

Since industrialization, urbanization and changing lifestyle started to demand for longer shelf life, the use of sorbate and propionic acid has been the main choice. Today's consumer, however, expect less use of synthetic preservatives but still they require food to be free from microbial growth, toxins, and other quality-deteriorating factors. Meanwhile, product freshness and sensor qualities must be preserved. The problem for the food industry is to fulfill these demands for minimum changes in food quality and maximum security.

So even though mold spoilage is a serious and costly problem for bakeries (1,23), the industry is looking for ways to reduce the amount of preservatives used. Several authors have shown that reduction of preservative concentrations to subinhibitory levels may stimulate growth of spoilage fungi (24,25) or/and stimulate mycotoxin production (26–29). It is therefore of interest to find alternative preservation methods for bakery products.

A. Organic Acid Preservatives

Among the most commonly used food preservatives are sorbates, benzoates, and propionates. Propionates have traditionally been the preferred preservative for bread; they inhibit molds and *Bacillus* spores, but not yeasts (30). Sorbate is more effective than propionate; it inhibits both molds and yeasts, and is used in a wide range of food products, including fine bakery products and confectionary (31); more recently sorbate has been included on the so-called positive list laid out by the European Commission Directive 95/EU/2 (32). Benzoates are used in many types of acidic food products, mainly fruit, and also used in combination with sorbate for confectionary.

Salts of the weak acid preservatives are more soluble in aqueous solution than the acid itself; therefore, the salt forms are preferred as food ingredients (33). Efficiency depends on the pH of the product, as the antimicrobial effect is much stronger in the undissociated form. The pKa values of propionic acid, sorbic acid, and benzoic acid are 4.88, 4.76, and 4.18, respectively, and maximum pH for activity is around 6.0–6.5 for sorbate, 5.0–5.5 for propionate, and 4.0–4.5 for benzoate (34,35). In a recent study of rye bread (pH 4.8) conducted in my laboratory, we concluded that growth of *P. roqueforti* could not be controlled by the maximum allowable amount of propionate (0.3%). *Eurotium rubrum* was inhibited but grew out at all conditions, whereas *P. corylophilum* and *P. commune* only grew at high water activity (0.97) and not at a_w 0.95 at the maximum propionate level (36). The same amount of sorbate was able to control growth. In a study of cakes, Marin and coworkers (37) found no growth at a_w 0.8 in the absence of preservatives, but growth was promoted when the weak acid preservatives were used in small amounts (0.03%). At a_w 0.85, pH 6.0, potassium sorbate was the most effective. It almost completely hindered growth of all fungi tested. At a_w 0.90, pH 6.0, neither benzoate nor propionate had an effect on fungal growth; only sorbate was able to inhibit fungal growth to some extent. Hence weak acid preservatives are able to extend shelf life of bakery products to some extent. Other alternatives must be considered, especially for products with pH close to neutral.

B. Sourdough

Rye bread, several wheat bread types, and some sweet baked goods have traditionally been fermented with sourdough. Besides improving the flavor and texture of the bread, this mixture of lactic acid bacteria and yeast increases the shelf lives of these products (38).

The antimicrobial effect of lactic acid bacteria has been assigned to both the lactic and acetic acids produced during fermentation (39,40); newer studies, however, have shown that lactic acid does not have an antimicrobial effect besides the pH-lowering effect (41–43). The antifungal effect of the dough seems to be more directly related to the acetic acid content (44). The antifungal spectrum of lactic acid bacteria varies, and antifungal effect has mainly been found in obligate heterofermentative *Lactobacillus* spp. Of these, *Lactobacillus sanfranciscensis* has shown the largest spectrum of antifungal activity (41). It produced a mixture of acetic, caproic, formic, butyric, and n-valeric acids, which synergistically inhibited species of *Fusarium*, *Penicillium*, *Aspergillus*, and *Monilia*. Caproic acid played a key role in inhibiting mold growth (41).

Lactobacillus sanfranciscensis is often isolated from sourdoughs in association with *L. plantarum*. This species has also been shown to produce antimicrobial compounds (43). These compounds corresponded to benzoic acid, 5-methyl-2,4-imidazolidinedione, tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one, and 3-(2-methylpropyl)-2,5-piperazinedione and were inhibitory to *Pantoea agglomerans* and also inhibited the fungus *Fusarium avenaceum* to some extent.

Another *L. plantarum* strain (21B) isolated from sourdough in Italy showed a very broad spectrum of activity against the most important bread-associated fungi (16). Spoilage by *A. niger* occurred after 2 days in breads started with *Saccharomyces cerevisiae* alone or with *S. cerevisiae* and the acidifying lactic acid bacterium *Lactobacillus brevis*, whereas fungal growth was not evident before day 7 on bread started with *S. cerevisiae* and *L. plantarum* 21B. *L. plantarum* 21B was found to produce a range of metabolites, of which phenyllactic acid was found to play a key role in inhibiting fungal growth.

Some sourdough cultures have also been proven effective against rope-forming *B. subtilis* and *B. licheniformis* spores. Rosenquist and Hansen (45) found that *B. subtilis* growth could be prevented by 0,1% v/w propionic or acetic acid or 15% *sourdough* fermented with *L. plantarum*, *Lact. brevis*, and *Lact. meltaromicus*. Katina and coworkers (46) found an almost similar effect of sourdough fermented with *L. palantarum* and *Peciococcus pentosaceus*. Here 20–30% sourdough could hinder rope formation.

C. Natural Preservatives

The antimicrobial properties of herbs and spices have been recognized and used for food preservation and medical treatments since ancient times (47,48). Scientific reporting on natural antimicrobial agents also dates back more than a century; Chamberlain reported in 1887 the action of essential oil vapors on anthrax spores, as cited by Maruzzella and Sicurella (49). A renewed interest in 'natural preservation' seems to be stimulated by present food safety concerns, growing problems with microbial resistance, and a rise in production of minimal processed food joined with 'green'-image policies of food industries.

Numerous studies have documented the antifungal (50–55) and antibacterial (56–58) effect of plant essential oils. Examination of indigenous and local herbs and plant material have been reported from all over the world, e.g., India (59) Australia (60), Argentina (61) and Finland (62).

Screening experiments with 13–52 essential oils and major active components against 5–25 microorganisms (63–67) have reported thyme, clove, cinnamon, bay, oregano, garlic, and lemongrass to be some of the best broad-spectra candidates for inhibition of food-borne pathogens and spoilage organisms. By comparing studies that have used different methodologies, it is clear that the minimal inhibitory concentrations (MIC) are highly dependent on the test method. Suhr and Nielsen (68) examined the application method and found large

differences between the antimicrobial effects, whether they were added to the media or added as a volatile to the packaging atmosphere. Figure 1 is a loading plot from a partial least squares (PLS) regression of degree of inhibition of fungal growth as affected by concentration and application method of a range of essential oils. Oils consisting of smaller volatile compounds were most efficient when applied through the gas phase. Mustard (1 ppm) and lemongrass completely inhibited growth of all fungi in the volatile system (marked with suffix V) for 14 days. Orange, thyme, sage, and rosemary A also strongly inhibited growth, but not equally well, all fungi.

Larger phenolic compounds worked better in direct contact (situated close to the upper right corner in Fig. 1). Thyme, clove and cinnamon almost completely inhibited growth for up to 14 days. Mustard (250 ppm) and to some degree lemongrass were also

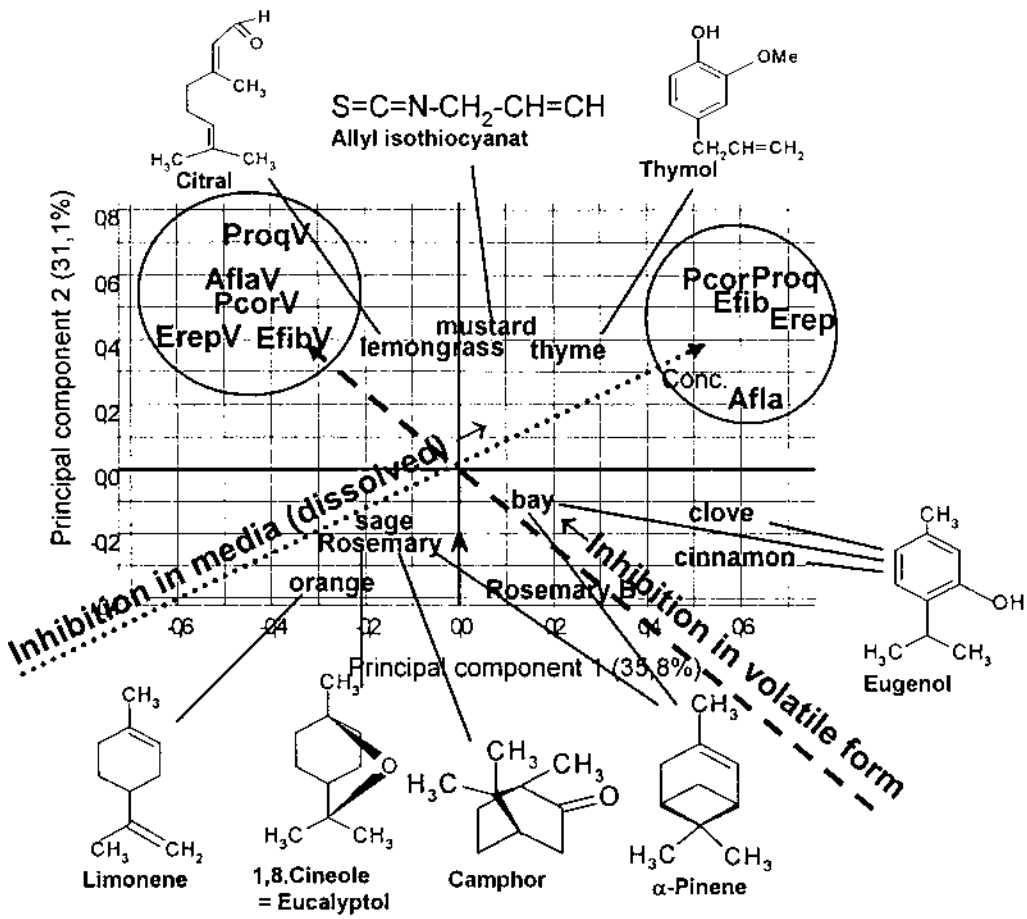

Figure 1 The inhibitory effect of some essential oils on the most important bread spoilage fungi: *Aspergillus flavus* = Afla; *Erotium repens* = Erep; *Endomyces fibuliger* = Efib; *Penicillium roqueforti* = Proq; *P. corylophilum* = Pcor as affected by application method. All oils were added in same amount in gas phase or in media (approx. 125 and 250 ppm) except mustard oil, of which only 1 and 2 ppm (μl/L) were added to the volatile gas phase (V). Oils situated closest to the upper left corner and the fungi with suffix V work well in a volatile system, whereas oils situated closest to the upper right corner work best when added to the media.

highly effective but the effect levelled off with time. These results indicate that mustard oil is inactivated when in contact with the media, because more than 250 times more had to be added to get the same effect in the media as in air.

For inhibition of mold growth on bread, smaller volatile compounds, such as allyl isothiocyanate and limonene, were most efficient when applied through the gas phase. Small volatile compounds are the most interesting for active packaging applications (see below) (69). Larger phenolic compounds, such as thymol and eugenol, were the best compounds for direct addition to the product.

V. PACKAGING

Most bread is packed just to keep it from drying out and to protect it against contamination. In that case, the typical packaging material is polyethylene because it is flexible and cheap. For the more advanced packaging solutions—like modified atmosphere packaging and active packaging with volatile antimicrobial compounds—more advanced foils are needed, which will increase the cost of these solutions.

A. Modified Atmosphere Packaging

In modified atmosphere packaging (MAP), the air inside the package is replaced with a single gas or a mixture of gases. The choice of gas atmosphere depends on the product to be packed, and for bread it usually consists of pure nitrogen (N_2), pure carbon dioxide (CO_2), or a combination of these. Other gases are only seldom used. N_2 is an inert, tasteless gas with low solubility in water and lipids. It has no antimicrobial effect but may be used to displace oxygen from the packaging atmosphere, thereby eliminating the basis for growth of aerobic microorganisms. CO_2 is mainly used in MAP due to is antimicrobial effect; however, it has to be used in relatively high concentrations (over 20%) to be effective (70,71). CO_2 is highly soluble in both water and lipid and the solubility increased with decreased temperature. The concentration of dissolved CO_2 will increase with increased partial pressure of CO_2 (concentration and pressure). When used in high concentration, it may therefore result in collapse of the package. This is especially the case in products with high moisture stored at decreased temperature (72). N_2 is often mixed into the gas atmosphere to avoid collapse of the package. The antimicrobial effect of CO_2 can be linked to the concentration of dissolved CO_2 in the product rather than the concentration over the product (73). This fact may be the main reason for the increased antimicrobial effect at reduced temperature that was reported for wheat bread (74). In order to secure a sufficient concentration of CO_2 in the product after packaging and to avoid collapse, it is important that the ratio between gas and product is not less than 2:1.

The antimicrobial effect of CO_2 is complex and four activity mechanisms have been identified (72): (a) alteration of cell membrane function, including effects on nutrient uptake and absorption, (b) direct inhibition of enzymes or decreases in the rate of enzyme reactions, (c) penetration of cell membranes, leading to intracellular pH changes, and (d) direct changes in the physicochemical properties of proteins.

The best performance in modified atmosphere packaging is obtained by the deep draw technique or similar methods that involves an evacuation of the package before it is flushed with the desired gas; however, this has a much lower throughput than flow pack (71). Flow pack results in a much higher oxygen level in the final pack because it is just forcing out the

air surrounding the product by blowing the package gas into the package just before it is sealed off. The spongy structure of bread thus results in residual oxygen content of 3–5%, which is insufficient to retard fungal growth (75). At this O_2 level, no effect of increased carbon dioxide concentration (25%) was seen on *P. roqueforti*, whereas it was somewhat inhibitory to *P. commune* (76).

Growth of one of the most important bread spoilage fungi (*P. roqueforti*) on the laboratory substrate CYA (69), packaged in modified atmosphere is plotted in Fig. 2. This shows that even at very low oxygen levels, this organism is able to reach a substantial size within 2 weeks. Carbon dioxide inhibits growth, but only by applying an oxygen scavenger could growth be completely inhibited. The yeast *Endomyces fibuliger* is even more resistant to low oxygen levels. It reached an average of 1.5 mm when cultured for 1 week in a package with an oxygen absorber, and 4 mm at 0.02 and 0.1% oxygen. Relative to the control, which reached 11 mm in one week, the diameter was 14, 36, and 36%, respectively (69). The effect of increased carbon dioxide was not tested for this fungus. In general, molds do not grow in packages with oxygen absorbers but at 0.02–0.03% residual oxygen in either pure nitrogen or 50% nitrogen and 50% carbon dioxide, their colony diameter reached 18–29% of the control. In pure carbon dioxide, the molds were strongly inhibited, reaching only 1–6% at 0.02–0.03% oxygen and 4–11% at 1.0% residual oxygen (69). Black and coworkers also found that oxygen levels lower than 0.2% were required to hinder fungal spoilage of pita bread (77). In MAP sponge cake, it was shown that growth of *Eurotium* was unaffected by changes in the residual oxygen level (0.02–0.5%) when the CO_2 level was high. At decreased CO_2 levels, however, the growth rate increased with increasing oxygen level (78). Several authors have discussed whether MAP will speed up the staling of wheat bread or not have any effect at all (79). In a recent study, Rasmussen and Hansen found that neither packaging in pure CO_2 nor in a mixture of CO_2 and N_2 resulted in a significantly different staling rate than storage in air (79). They found that loss of water was the most important factor besides the crystallization behavior of starch in the bread. Thus, MAP could be used to extend the microbial shelf life of wheat bread without risk of speeding up staling (79).

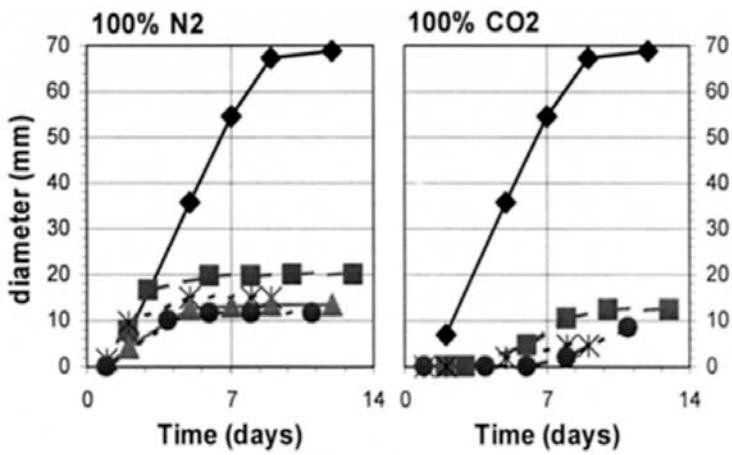

Figure 2 Growth of the most important spoilage organism on rye bread, *Penicillium roqueforti*, at 25°C on a laboratory growth substrate, CYA at pH 7.0: ♦ Air, ■ 1.0% O_2, ∗ 0.5% O_2, ▲ 0.1% O_2, ● 0.01% O_2.

B. Active Packaging

Active packaging, AP, may be an interesting alternative to both the traditional use of preservatives and MAP. The term active packaging is used to describe a quite large group of packaging types that involves interactions between package components and the internal gas atmosphere, or the food itself, during storage. The main objective is to control or react to events taking place inside the package in order to improve shelf life or indicate quality.

One type of AP is also termed interactive packaging because it actively interacts with the food by removing or adding substances to the internal gas atmosphere in the package or to the food itself. Best known are the oxygen absorbers, which within a short time after the product is packed will have reduced the oxygen content in the package down to a level at which most aerobic microorganisms will stop growing and product oxidation ceases or in some cases even reverts (81). Other absorbers remove carbon dioxide, moisture, off-flavors, or ethylene that otherwise would speed up maturation of fruits and cut flowers. The substances that can be added are antimicrobials, antioxidants, or flavors and, in some cases, color.

Intelligent, smart, or clever packaging is a type of AP that contains an indicator of product history or quality. Best know are the time temperature indicators, which indicate the remaining shelf life of products, based on how they have been stored and transported. More interesting for bakery products are the indicators that can show if the oxygen level is sufficiently low, or the carbon dioxide level is high enough, as they will also indicate if the package is leaking. Some can even indicate spoilage or quality by reaction to volatile substances from spoilage reactions in food.

1. Oxygen Absorbers

The most widely researched and patented area of active packaging is the use of oxygen-absorbing systems (80–83). Since the first iron-based pouch-type oxygen absorber was introduced to the market in Japan in 1977, there has been a sharp increase in designs and applications of oxygen absorbers, first in Japan and then also in the United States and last in Europe (82). The most widespread type is based on powdered ion; others are based on ascorbic acid, glucose oxidase, alcohol oxidase, and ethylenically unsaturated hydrocarbons. The most common form of oxygen absorbers is the sachet. The active component is sealed into a small sachet that is very permeable to oxygen. At the packaging line these sachets are introduced into the package and it is sealed off. It may also be introduced in the form of a label, which is stuck on the inside of the pack. This has a great advantage: the absorber can be placed in a way that it is less obvious for the consumer and the absorber will not fall out when the package is opened. Recently, packaging materials with the oxygen absorber integrated into the film have been introduced.

The most important advantage of oxygen absorbers is that they are able to reduce the headspace oxygen content inside the package down to ultra-low levels (0.01 %) that cannot be obtained in commercial packaging lines. Moreover, if the absorber has a sufficient capacity, it will absorb all the oxygen that may have been trapped inside the food and that which permeates though the film or leaks through pinholes into the headspace during storage.

It is also an advantage that it is relatively cheaper to set up a packaging line with gas absorbers than with gas packaging; however, the running costs are somewhat larger and the sachets or labels are visible to the consumer.

Several investigations have shown that mold growth on bakery products can be avoided for a very long time using oxygen absorbers (69,77,84,85). These papers indicate

that as long as the non-oxygen-related chemical changes, like staling, are at an acceptable level, the limiting factor is the absorbing capacity of the absorber in combination with the oxygen permeability of the package. For military use, shelf life of over a year may be necessary (84), whereas in most other cases a shelf life of more than a month is not required. Oxygen absorbers are very efficient at inhibiting mold but several authors have shown them to have a limited inhibitory effect on yeast. Black and coworkers (77) found that the yeast count per gram of pita bread increased from less than 10 to 3100 during a 28-day storage, even though it was still not visible.

2. Antimicrobials

In a recent review of antimicrobial food packaging, Appendini and Hotchkiss (86) point to antimicrobial materials as an area that is expected to grow in the next decade. Several of the antimicrobial compounds from spices, herbs, and fruits that were mentioned in Sec. IV.C may find use in active packaging. Although different results are observed depending on test conditions, microorganisms and source of the antimicrobial compound, some spices or essential oils always act very effectively in inhibiting growth. One of these is mustard essential oil, which primarily contains the active compound allyl isothiocyanate (AITC), which even at low doses is very active in inhibiting growth of most microorganisms (87). When fungi are exposed to sufficiently high concentrations of volatile AITC, it will not only inhibit the growth like most antimicrobial compounds but also efficiently kill the fungi (69,88). Therefore, the product may stay mold-free even if most of the active component is loss through the packaging material during storage. This is a great advantage when this compound is used for active packaging. AITC is commercially available as labels in Japan under the name Wasoouro (89); however, it has still not been approved elsewhere (69,86).

In general, the authorities consider antimicrobials that may migrate into the food as preservatives, thus they have to meet food-additive standards. Therefore the use of substances like AITC may be prohibited even though it is allowed as a flavor agent in much higher doses (69).

3. Alcohols

A very well known antimicrobial is ethanol, which has been used for disinfection and sterilization worldwide. Among all the alcohols, ethanol is the most widely used and studied in relation to antimicrobial effect. Ethanol may be added to the packaging atmosphere by an ethanol vapor generator consisting of ethanol absorbed or encapsulated in a carrier material and enclosed in polymer packets. From this packet, ethanol is released into the packaging atmosphere. The amount of ethanol that can be released is relatively low so this method has primarily been used for products with a_w of less than 0.92 (90). In general, alcohols are found to inactivate many vegetative microorganisms but their effect on spores is less strong (91). especially, ascospores, are highly resistant (20); therefore, growth of *Eurotium* is expected to be a problem in these products. Another drawback is the characteristic off-flavor of ethanol.

VI. OTHER METHODS TO EXTEND SHELF LIFE

To extend the shelf life of rye bread, some factories heat the packaged bread to 70°C in a continuous microwave heater (8). This will kill most of the fungi associated with the bread and extend the shelf life several days. However, this treatment may result in development of off-flavors and changes the texture of the bread. An overview of the fundamentals of

microwave heating, its equipment and applications is given by Ohlsson and Bengtsson (92). Ionizing radiation is also a possible alternative for extending shelf life. The effectiveness and safety of irradiation is well documented and is recommended by the World Health Organisation (WHO) for treatment of a wide range of foods. However, the method is expensive to set up and to run (93,94). Besides, public opinion in Europe is very much opposed to irradiation.

VII. CONCLUSIONS AND PERSPECTIVES

Spoilage of bakery products is mainly caused by fungal growth. Several methods for controlling spoilage have been presented. Most important is proper hygiene and the implementation of a HACCP plan where the focus is not placed only on the end product but on the whole chain: from the producer of raw materials (mycotoxin-free grains) through the production, transportation to storage, and handling at retail stores. There is a great demand for removal of preservatives from bakery products; one alternative to preservatives is the use of modified atmosphere packaging. It has already proved to be an effective method for protecting bakery products and is widely used to extend shelf life of bakery products. However, it is a relative costly method and it alone will in some cases not completely hinder fungal spoilage. Therefore, active packaging with oxygen absorbers could be an alternative for today, and active packaging using natural preservatives could be the solution of tomorrow.

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44

Kenkey: An African Fermented Maize Product

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I. INTRODUCTION

Kenkey is an indigenous fermented product commonly produced in Ghana, West Africa, especially in the coastal areas. Traditionally it has been produced by two ethnic groups, the Gas in the Greater Accra Region and the Fantis in the Central and Western Regions. There are therefore two types of kenkey, Ga-kenkey (also called *Komi*) and Fanti-kenkey (also called *dokono*). Slight differences exist in the organoleptic quality and the processing procedure for the two types of kenkey. Both are produced by the fermentation of maize dough into a sourdough that is subsequently cooked—wrapped in maize husks in the case of Ga-kenkey or plantain leaves in the case of Fanti-kenkey. The Fanti-kenkey is fermented for a slightly longer period than the Ga-kenkey, and salt is also added to the latter during processing.

Maize is the principal cereal produced in Ghana and is consumed as a staple crop in most parts of the country. Its annual production is currently 1,034,200 metric tons, accounting for 3% of the Agricultural Gross Domestic Product. The bulk of the maize produced is consumed in the form of kenkey. The processing of maize into kenkey is an important commercial activity undertaken mainly by women. Kenkey is consumed principally in the coastal areas. It is consumed as a main meal served with fried or grilled fish and an accompanying sauce or soup. The sauce is usually a blend of onions, tomatoes, pepper, and salt, which is freshly ground and uncooked. It forms an important article of diet in the food-eating habits of low-income workers who may eat it as breakfast, lunch, or dinner. Kenkey is a heavy meal because it is bulky, so when eaten as breakfast, it carries through to dinner thus making it economical. It may also be mashed with water into a thin porridge, a sort of refreshing drink to which sugar and milk are added.

II. COMMERCIAL PRODUCERS AND SOCIOECONOMIC IMPORTANCE

The process of kenkey-making is lengthy and laborious, therefore it is more often purchased from a commercial kenkey producer rather than cooked at home. The producers–

who are mainly women with little or no formal education–carry out commercial production as a family-acquired art. The small-scale processors carry out their activities either as individuals or as a family business in the household often depending on family labor to produce and retail the product.

A survey conducted in Accra in 1996 (1) found that at most production sites the amount of maize processed weekly ranged from 0.05 to 1.2 metric tons with an average of 0.3 tons of maize processed into 0.5 tons of kenkey. There are however, a few large production sites with weekly capacities of several tons (up to 5 tons) of maize. Such large production sites do not only produce kenkey for sale but also sell the intermediate product, the fermented dough, which is also used to prepare other products such as *koko* and *banku*.

The production of kenkey is based on traditional technologies, that have been handed down in generations. Production costs, apart from the raw material, maize, are minimal because the family labor employed is often not perceived as costs. This makes the product affordable, providing food for a large part of the urban population especially the low-income group.

III. NUTRITIVE VALUE AND IMPORTANCE IN THE DIET

The nutritive value of kenkey is basically dependent on the maize from which it is made and the processing technique used in production. Maize contributes significantly to the total calorific and protein content of the diet of people who consume it as a staple in Ghana and is richer in protein than other staples such as cassava, coccoyams, yams, and plantain. However, the traditional maize varieties are deficient in lysine, tryptophan, and B vitamins (2). It has been estimated that maize accounts for 90–95% of the total calories and over 70% of the dietary proteins of some people in parts of the coastal areas (3).

On a dry-matter basis the proximate composition of Ga-kenkey is roughly 8.9–9.8% protein, 1.3–3.2% fat, 0.5–1.9% ash, 10.6–78.6 mg/100g calcium, 202.4–213.8 mg/100 g phosphorus, 6.5–12.6% mg/100 g iron, and 74.3–87.1% total carbohydrate (4–7).

IV. TRADITIONAL METHOD OF PRODUCTION

The traditional method of production is shown in the flow diagram in Fig. 1 and involves cleaning, steeping, milling, dough fermentation, *aflata* preparation, mixing of *aflata* and raw dough, molding and packaging, and cooking into kenkey. A traditional production site is illustrated by Fig. 2 and a partly upgraded site by Fig. 3.

A. Cleaning

Maize for processing into kenkey is cleaned to remove all foreign matter. This is done by one or a combination of several processes including winnowing, hand picking, sieving, and sedimentation. These operations remove dust, chaff, stones, insect-damaged grains, and other debris. The sedimentation process involves pouring the grains into a big basin of clean water, stirring with a wooden ladle to allow the mature and good-quality grains to settle at the bottom while the less dense immature, some insect damaged, and diseased grains float on the surface. The latter are collected with small baskets or sieves and used as animal feed. The good maize is then washed again in water before steeping.

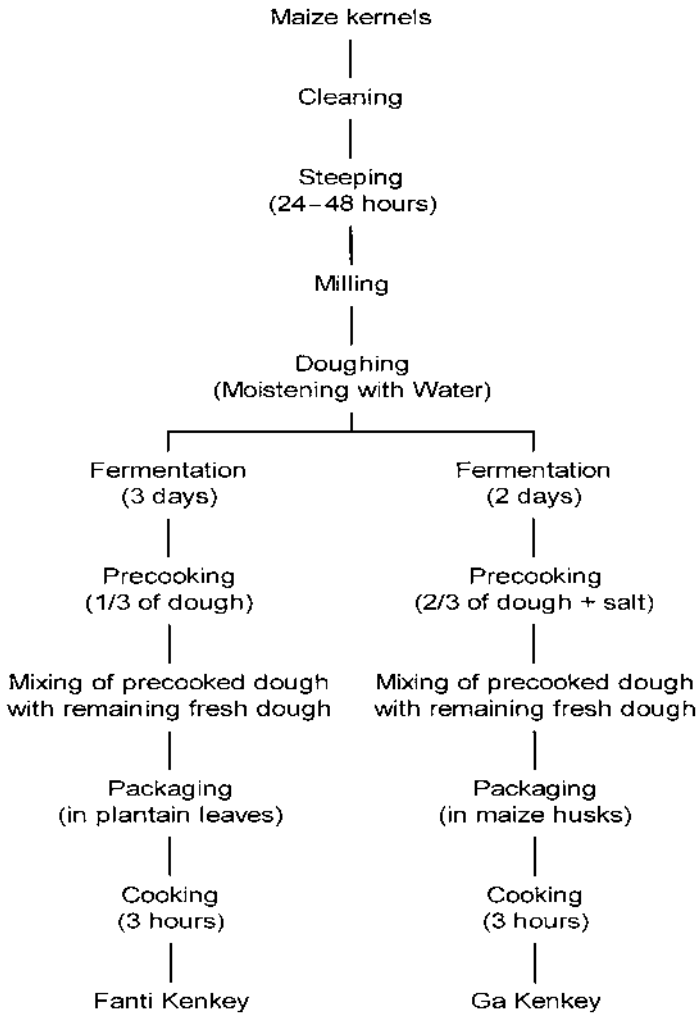

Figure 1 Flow diagram showing the various steps of fermentation of maize for production of kenkey.

B. Steeping

This involves the steeping of the maize grains in clean water for 1 to 3 days depending on the initial moisture content and hardness of the grains. Most local varieties are steeped for 1 day, but some hybrid maize with very hard kernels and high portion of testa are steeped for up to 3 days to soften the kernels and facilitate milling. The steep water is drained off and the maize washed before milling.

C. Milling and Doughing

The steeped maize is milled in a plate mill popularly known in Ghana as corn mill into a very smooth meal; which is then mixed with water to form a dough with a moisture

Figure 2 Traditional site of maize fermentation in Accra, Ghana. The figure shows a fermentor in the back, the molding of balls of the mixture of fermented and unfermented dough, and wrapping of the balls in maize husks.

content of about 50–55%. The amount of water used to form the dough is very important as this affects the rate of fermentation as well as the quality and shelf life of the dough. This amount varies widely from one producer to another, between 17 and 44 liters of water to 100 kg of maize (1).

D. Fermentation

The dough is packed tightly into wooden vats, aluminum pots, enamel or aluminum basins, plastic containers, and so forth and allowed to ferment spontaneously for up to 3 days at ambient temperature (i.e., 25–30°). The size of the fermentor is variable but it will normally not exceed 50 kg of dough. Normally, after 2 days of fermentation, the dough is ready for use in making different products including kenkey, *banku*, and *koko*. Dough fermented for 24 hr does not give a good-textured product, but this can be mixed with older dough to obtain the desired texture. Sometimes the traditional processors hasten the fermentation by backslopping with old dough. With back slopping, fermentation can be shortened to 24 hr. Usually the processors use up all the fermenting dough between the second and third day, but if fermentation is prolonged beyond the third day it might lead to the development of undesirable flavors and high acidity. However, according to some commercial producers, the shelf life of the dough can be extended without adverse effect on consumer acceptance if less water is used in making the dough.

Figure 3 An upgraded site for maize fermentation. The figure shows the fermentors made of aluminum.

The dough may also be sun-dried after the desired acidity has been obtained within 2 days.

E. Aflata Preparation and Mixing

A portion of the fermented dough is made into a slurry by adding two or three parts of water and cooked with continuous stirring into a sticky gelatinous paste known as *aflata*. During this process, salt is added in the case of Ga-kenkey. The *aflata* is mixed thoroughly with a portion of the uncooked fermented dough using wooden ladles and allowed to cool. The ratio of *aflata* mixed with uncooked dough depends on the type of kenkey being produced and the preference of the consumers. Amongst the indigenous *Ga* people who consume kenkey as a major staple, the ratio of aflata to the uncooked dough is usually 1 to 1. However, some producers mix two-thirds of *aflata* with one-third of the uncooked dough, and others mix one-third *aflata* with two-thirds uncooked dough. The ratio of aflata mixed with the uncooked dough determines the texture of the kenkey that will be produced. In a sensory evaluation of the texture of kenkey, the highest score was given by panellists for kenkey prepared from a 1-to-1 *aflata* to uncooked dough mixture (8). The study confirmed that *aflatalisation* is necessary to produce kenkey of the desired texture. *Aflata* is reported to act as a binding agent when mixed with uncooked fermented dough and enables the product to be molded into balls and other shapes (9).

F. Molding and Packaging

For Ga-kenkey, the mixture is molded into balls of uniform sizes of about 300 g weights and wrapped in clean prewetted maize husks. Fanti-kenkey is molded into cylindrical shapes and wrapped with plantain leaves.

G. Cooking

Some clean maize husks or plantain leaves are placed at the bottom of large aluminum cooking pots and the balls of kenkey are packed on top to prevent the balls from sticking to the pots during cooking. Boiling water is poured into the pot to cover the balls and the top covered with a piece of cloth or polythene sheet to conserve steam. The kenkey is cooked for about 3 to $3\frac{1}{2}$ hours. The length of cooking depends on the ratio of *aflata* to the uncooked dough and on how well the *aflata* was cooked. For kenkey containing less *aflata*, boiling lasts longer. After the balls are well cooked, they are removed from the pots and placed in large bowls lined with polythene sheets, which are also used to cover the balls of kenkey to keep them hot until they are all sold for consumption. The cooking water left in the pot, referred to as kenkey-water is collected and drunk as a thin porridge and is believed to have curative properties against malaria, diarrhea and jaundice. It has been reported that the carbohydrate and electrolyte levels of kenkey water are comparable to the UNICEF/WHO Oral Rehydration Salts and therefore suitable for use in oral rehydration in Ghana (10).

V. MICROBIOLOGY OF FERMENTATION

In kenkey production, fermentation occurs during the steeping of maize and the fermentation of the dough. Earlier studies on the microbiology of maize dough fermentation during kenkey production carried out in 1970, reported the presence of a mixed population of lactic acid bacteria and yeasts at the advanced stage of fermentation (11). The bacteria consisted of homofermentative *Pediococcus cerevisiae* and the heterofermentative species *Leuconostoc mesenteroides* and *Lactobacillus fermentum*. Other investigators (12) identified the dominating lactic acid bacteria in spontaneous fermentations of whole maize meal under laboratory conditions as heterofermentative *L. fermentum*, *Lactobacillus cellobiosus*, and *Pediococcus acidilactici*.

In recent years, detailed studies have been carried out on the microbial ecology of kenkey manufacture at two large commercial production sites in Accra and also under laboratory conditions (13). The studies showed that at the start of maize steeping, a mixed group of microorganisms are present, including lactic acid bacteria, gram-positive catalase-positive bacteria, gram-negative bacteria, yeasts, and molds. During steeping, a strong selection takes place leading to a significant increase in the population of the lactic acid bacteria and a reduction in the population of the catalase-positive bacteria. After milling and addition of water, the main fermentation starts and within 0–72 hours the lactic acid bacteria multiply to levels of more than 10^9 CFU/g. Yeast growth reaches a maximum level of 10^6 CFU/g after 24–48 hr, followed by a decline in numbers. Filamentous fungi present reduce significantly and after 24 hr of dough fermentation, molds and catalase-positive bacteria are not detected at levels of 10^2 CFU/g. No explanation has so far been offered on this apparent inactivation of filamentous fungi. At the

advanced stage of fermentation, the microbial population consists only of lactic acid bacteria and yeasts. Of 343 representative isolates of lactic acid bacteria taken from 15 samples, 95% were found to be obligately heterofermentative lactobacilli whose pattern of carbohydrate fermentation was closely related to *L. fermentum* and *Lactobacillus reuteri* (13). Twenty-nine of the remaining isolates were found to be facultative anaerobic homofermentative cocci, which were identified based on their biochemical profiles as *Pediococcus pentosaceus* and *P. acidilactici*.

In a later study (14) involving molecular characterization of the dominant lactobacilli isolated from the two production sites, 172 isolates were characterized by use of Random Amplified Polymorphic DNA (RAPD) and grouped into clusters based on their RAPD profiles. The RAPD method identified the dominant lactic acid bacteria as *L. fermentum* by dividing the isolates into two main clusters: cluster 1 included *L. fermentum* and cluster 2 the remaining *Lactobacillus* species. Furthermore, cluster 1 was divided into four main subgroups allowing for strain differentiation and subspecies typing. Within the *L. fermentum* group, two distinct bands of approximately 695 bp and 773 bp were common to all strains, including reference strains of *L. fermentum* included in the study. The two conserved RAPD fragments were cloned, sequenced, and used as probes in southern hybridization experiments. By examination with other *Lactobacillus* spp. the probes hybridized exclusively to EcoRI digested chromosomal DNA of *L. fermentum* strains at different lengths, thus indicating the specificity of the probes and variation within the *L. fermentum* isolates (14). Sub species typing of the isolates showed that a variety of *L. fermentum* strains occurred on the maize, and additional strains were added from steeping tank and corn mill, and that the varieties of strains remained throughout the fermentation. There was no selection for any particular strain during the fermentation process.

Some authors have reported the dominance of *Lactobacillus plantarum* in the later stages of maize dough fermentation during kenkey production (15,16). In whole maize dough and maize dough supplemented with 20% white cowpeas, *Lactobacillus confusus* was reported as the dominant lactic acid bacterium present at the initial stage of fermentation. However, later it was succeeded by *Pediococcus* species and *L. plantarum* for whole maize and mixed maize and white cowpea doughs, respectively. In mixed maize and red cowpea dough, *L. plantarum*, *L. confusus*, and *Pediococcus* spp. were found in similar proportions throughout the 4-day fermentation period (15). In studies (16) on selected African fermented foods, 48 *Lactobacillus* isolates from kenkey were obtained; which were identified as *L, plantarum*, *L. fermentum*, *Lactobacillus brevis*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus cellobiosus*. *L. plantarum* was reported as the dominant organism, but the proportions in which it occurred was not indicated (16).

An investigation of the yeasts and molds present during the fermentation of maize dough has reported a mixed population comprising *Candida*, *Saccharomyces*, *Trichosporon*, *Kluveromyces*, and *Debaryomyces* species in the raw maize, during steeping, and in the early stages of fermentation (17). A selection was observed during steeping and maize dough fermentation, with *Candida krusei* and *Saccharomyces cerevisiae* becoming the dominating yeasts, with counts exceeding 10^6 CFU/g. Other workers have also found *S. cerevisiae* and *Candida* spp. to be the dominant yeasts in maize dough fermentation during kenkey making (13,18). It has been suggested that the yeasts influence the organoleptic and structural quality of the dough because inoculation of maize dough with 10^6 CFU/g of *S. cerevisiae* in pure cultures or in combination with various *Candida* spp, increased the organoleptic scores of the dough significantly (19).

The species of the dominant yeasts involved in the fermentation of maize during processing into kenkey have been confirmed by molecular methods (20,21). Determination of chromsome profiles and polymerase chain reaction (PCR) profiles of 48 isolates of *S. cerevisiae* showed that these isolates could be grouped into clusters (20). In the same study, four different *MAL* genotypes were observed, with *MAL11* and *MAL31* predominating. *MAL11* was seen for all isolates, whereas no evidence of *MAL21* and *MAL41* was observed. *MAL* refers to a locus of the genome responsible for the regulation of the uptake and hydrolysis of maltose, the main fermentable carbohydrate of maize dough. A total of five different *MAL* loci are described for the genome of *S. cerevisiae* (20). Phylogenetic studies of *S. cerevisiae* isolated from different food and beverage fermentations including fermented maize show characteristic patterns of *MAL* loci (i.e., *MAL* genotypes). The studies showed that a high number of *S. cerevisiae* isolates were involved throughout the spontaneous fermentation of maize dough (20).

With respect to *Candida kusei* chromosome profiles, PCR profiles, restriction endonuclease analysis (REA), and Southern blot hybridization techniques revealed DNA polymorphism that enabled subspecies typing of 48 isolates of *C. krusei*. On the basis of PCR and REA profiles, isolates were grouped into clusters. The results showed that several strains of *C. krusei* were involved in the fermentation of maize dough from the onset and remain dominant throughout the fermentation (21).

The presence of molds during the initial stages of maize dough fermentation has also been reported (17). These molds—*Penicillium*, *Aspergillus*, and *Fusarium* species, including potential mycotoxin producers—were isolated from raw maize, but during the maize dough fermentation, the initial high counts of 10^5 CFU/g were reduced to less than 10^2 CFU/g within 24 hr of fermentation. As mentioned earlier, no explanation has been offered of the strong fungicidal effect in fermenting maize dough.

VI. ANTIMICROBIAL INTERACTIONS AND FOOD SAFETY ASPECTS

In a study of antimicrobial interactions involving 241 isolates of *L. plantarum*, *P. pentosaceus*, *L. fermentum*/*reuteri*, and *L. brevis* isolated from various stages of processing during kenkey production, widespread microbial interactions were observed between the maize isolates, amounting to 85% of all combinations tested (22). For the steep water, 41% interactions were observed; for the fresh dough, 35% interactions; and for the fermented dough, 18% interactions. Each processing stage was shown to have its own microenvironment with strong antimicrobial activity. These interactions were suggested to explain the microbial succession that takes place among the lactic acid bacteria during the processing of maize into kenkey. The antimicrobial effect was explained to be mainly due to the combined effects of acids, compounds sensitive to proteolytic enzymes, and other compounds with antimicrobial activity with acid production being the most important factor (22).

With respect to molds during maize fermentation, inhibitory effects of *C. krusei* and *S. cerevisiae* have been demonstrated against isolates of *Penicillium citrinum*, *Aspergillus flavus*, and *Aspergillus parasiticus* isolated from maize as well as 15 known mycotoxin-producing strains of the same mold species. The *Candida* isolates showed greater inhibitory potential than the *Saccharomyces* isolates and the *Penicillium* species were more sensitive to the yeasts than the *Aspergillus* species. The mold growth was not affected by either yeast supernatant or cell-free extracts, suggesting that the inhibitions were not due to compounds produced by the yeasts and were attributed to competition for nutrients between the yeasts and the molds (23).

Earlier work (24) carried out on the antimicrobial effect of fermented maize dough on the pathogen *Shigella flexneri* and enterotoxigenic *Escherichia coli* showed that although unfermented maize dough could not inhibit growth of any of these pathogens, fermented maize dough inhibited half of the strains tested when examined 8 hrs after inoculation. The cooking of the fermented dough into porridge reduced the antimicrobial effect despite the acid pH of 3.3, and it was suggested that the antimicrobial effect of fermented maize dough was not due to pH per se but involved some unique heat-labile antimicrobial compound (24).

Investigations have also been conducted on the hygienic safety of kenkey as a fermented microenvironment by monitoring the survival rates of selected pathogens enterotoxigenic *Escherichia coli* J955 and 101685, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The populations of the test bacteria were reduced by 1–3 log units in 30 min in Ga-kenkey of pH 4 but the rate of decline was variable for different organisms. A similar trend was observed for kenkey-water (7).

The antimicrobial properties observed for lactic fermented cereal foods have been explained to be largely due to acid production by lactic acid bacteria as well as other antimicrobial factors (22). These antimicrobial factors include bacteriocins, low pH, carbon dioxide, hydrogen peroxide, ethanol, diacetyl, low redox potential, nutrient depletion, and crowding (25). Four strains of *Lactobacillus plantarum* isolated from kenkey were subjected to bacteriocin assay and reported to produce bacteriocin (26,27). However, the extensive biochemical characterization of the bacteriocin has not been reported.

Isolates of dominant *Lactobacillus fermentum* from fermented maize dough have also been investigated for their probiotic characteristics (28). Twenty-one isolates were tested for tolerance to pH 2.5, sensitivity to the toxic effect of bile salts, antimicrobial activity, and adhesion to Caco-2 cell lines. The strains did not show significant growth at pH 2.5 but maintained high cell viability. High viability and weak growth was observed for bile salts. Antimicrobial activity varied among the strains but 90% of the strains inhibited the growth of *Shigella flexneri*, 47% inhibited *Salmonella typhimurium*, 42% *Escherichia coli*, 19% *Bacillus cereus*, and 19% *Listeria monocytogenes* under conditions of low organic acid production indicating the presences of antimicrobial substances other than acid. For their adhesion potential, 34% of the strains had the property to adhere to Caco-2 cells; however, they were not strongly adhesive compared to positive adhesive strains included as reference. For two strains, verification of probiotic characteristics was carried out in a pig-feeding trial. The results obtained showed that one strain did not survive the maize fermentation process, whereas the other was recovered as the dominant *Lactobacillus* species in the intestinal tract of the pigs. This study indicated a rich source of strain variability in the microflora of maize dough in which strains with probiotic characteristics could be selected.

VII. BIOCHEMICAL AND NUTRITIONAL CHANGES DURING FERMENTATION

During kenkey production, the pH reduces from about 5.9 to 4.2 during 24 to 48 hours of steeping. After milling and addition of water, the pH increase to about 5.3 but drops rapidly during fermentation to 3.7 after 24 to 48 hours (13). As the reduction in pH is observed, an increase in titratable acidity is also recorded from about 0.7 to 2.2 mg NaOH g^{-1} after 2 days of maize dough fermentation (29). The main carboxylic acids produced during the fermentation of maize dough are D + L-lactic acid and acetic acid in

concentrations of 0.8–1.4% and 0.1–0.16%, respectively. Other acids produced are propionic and butyric acids with values of 30 mg kg^{-1} and 40 mg kg^{-1}, respectively. A volatile: nonvolatile acids ratio of 0.16 is required to produce the acceptable degree of sourness for fermented maize dough (13,29).

A complex combination of factors during steeping, milling and dough fermentation contribute to the final pasting characteristics of kenkey (30). Endogenous enzymatic activity, hydration, and grain softening during soaking combine to facilitate the release of starch during milling, thus ensuring better hydration and swelling of granules, to achieve a high degree of gelatinization and set back viscosity necessary for good *aflata* quality. A dough with a high starch gelatinization index (during cooking) and a high set back viscosity on cooling is required to give an *aflata* of adequate binding and moisturizing capacity important in determining the desired textural characteristics of cooked kenkey (30).

In one study, it was reported that steeping of maize for 1 to 2 days decreased the total dietary fiber content by 40%, 46%, and 53% for maize, maize-cowpea (var. Asontem), and maize-cowpea (var. Benpla) mixtures, respectively. In vitro protein digestibility also increased by 32%, 25%, and 18%, respectively (31).

The fermentation of maize during processing into kenkey may improve the nutritional status of the product by increasing synthesis of B vitamins (e.g., thiamine), protein digestibility, and bioavailability of nutrients, among others. It has been reported that soaking of maize and maize-cowpea blends resulted in significant increases in lysine availability by about 20% for maize and maize-cowpea mixtures (32). Cooking of soaked samples further improved lysine availability by 68% and 31% for maize and maize-cowpea mixtures, respectively. Fermentation for 2 days further increased these values by an additional 22% and 12% for maize and maize-cowpea doughs. Prolonged fermentation and cooking effected further significant improvements in lysine availability. Maize and maize-cowpea doughs fermented for 4 days and cooked for 3 hr achieved values of 3.42 and 4.43 g/16 g N, respectively (32).

The increased availability of essential amino acids resulting from lactic acid fermentation may be related to a reductoin in proteinase inhibitors (e.g., trypsin inhibitor) in legumes and a reduction of tannins (33,34). Lactic acid bacterial strains isolated from kenkey and other Ghanaian fermented foods have been found to show different abilities to degrade or inactivate trypsin inhibitor under defined conditions (35). *Lactobacillus plantarum* strain 91 and *Leuconostoc* sp. 106 isolated from kenkey were able to degrade trypsin inhibitor by about 50%.

Very little or no change occurs in the level of riboflavin in maize dough during fermentation, whereas cooking maize dough into *banku* and Ga-kenkey results in a mean loss of 33% and 36% riboflavin, respectively (36,37). The thiamine content, however, is reported to increase considerably during fermentation of maize dough from 339.1 μg/100 g maize to 389.3 μg/100 g fermented dough (37).

VIII. UPGRADING OF TRADITIONAL TECHNOLOGY AND USE OF STARTER CULTURES

Several studies have been carried out to upgrade and mechanize some of the unit operations involved in the production of kenkey, including the development of shelf-stable dehydrated fermented maize meal flour as a convenience intermediary product. As mentioned above, a partly updated traditional production site is shown in Fig. 3.

Spontaneously fermented maize dough has been successfully dehydrated in a hot air tray dryer (Fig. 4) at temperatures of 60°C, 120°C, and 200°C to produce acceptable

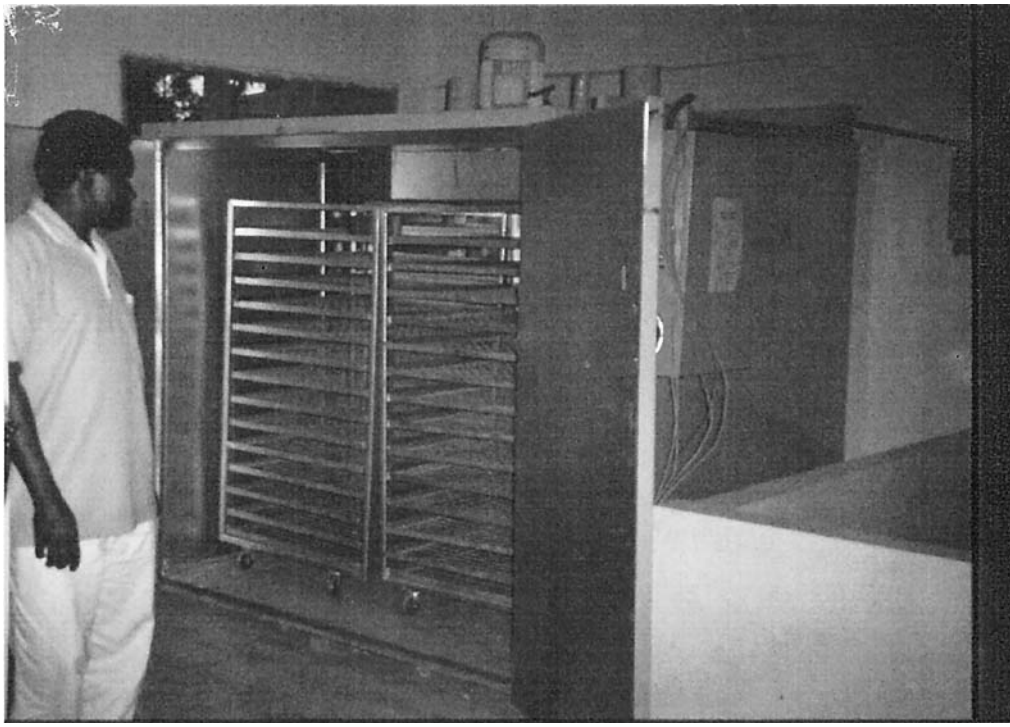

Figure 4 A hot air dryer for dehydration of fermented maize dough. (CSIR—Food Research Institute, Accra, Ghana.)

product (38). Dehydration at a temperature range of 60–70°C did not affect total acid content of the product, and taste panel evaluations found products such as *akasa*, *koko*, and *banku* made from the dehydrated dough acceptable. Dehydrated fermented maize meal made by this method can be reconstituted into fresh dough by adding water and is now produced on commercial basis for sale in Ghana and also for export.

An accelerated option for industrial production of kenkey flour was developed by inoculating dry-milled maize flour with dough containing an enrichment of lactic acid bacteria to accomplish fermentation within 24 hr of incubation at 30°C to obtain the required level of acidification (15). Subsequently, the dough was dehydrated into kenkey flour and pregelatinized *aflata* using cabinet and drum drying. Although the two methods were effective in preparation of pregelatinized *aflata*, drum drying caused a 34% reduction in titratable acidity of the fermented dough whereas cabinet drying had a less drastic effect. The possibility of using a mixture of drum-dried *aflata* and uncooked cabinet dried flour for convenient preparation of kenkey at domestic level was demonstrated. However, dry-milled maize flour had inferior pasting and setback viscosities as compared to the traditionally prepared dough and was not suitable for the production of pregelatinized *aflata*. The study concluded that even though dry-milling and accelerated fermentation of dough could drastically reduce kenkey production time from 6 days to within 24 hr, without steeping a product of inferior texture is obtained.

Another option for the industrial manufacture of kenkey in sausage casings, which takes approximately 24 hr, has also been developed (39). This method involves precracking the kernels to reduce hydration time of maize from 48 to 10 hr, incorporating *aflata* into the dumpling prior to fermentation to reduce fermentation time to 12 hr, and

the use of a starter dough. The cooking time and energy expenditure was reduced from 2 hr to 35 min by changing the dimensions of the kenkey balls from 10–15 cm diameter to 6 cm diameter cylinders. Due to the different processing conditions, yeasts were found to be more active than in traditional fermentation, resulting in higher alcohol levels. However, these alcohol levels remained low and ethanol disappeared after cooking. The combination of lactic acid fermentation and cooking resulted in a microbiologically stable product even after the dumpling had been deliberately contaminated.

This study concluded that the traditional kenkey-making process could be shortened to 24 hr by a combination of reduced steeping time, use of starter dough in a dough-*aflata* mixture, and packaging in sausage casings. However, it should be noted that the traditional packaging of kenkey in maize husks or plantain leaves gives it a unique sensory characteristic that consumers associate with the product.

Attempts have also been made to increase the protein content of kenkey by fortification of the dough with amino acids, soybeans (40,41), cowpeas (42) and also by the development of Quality Protein Maize (QPM) varieties (4,5). In addition to increasing the protein content, addition of boiled whole soybeans to soaked maize before milling and fermentation reduced the fermentation time by 60% (41).

A lactic acid bacteria–enriched starter dough has been developed by back-slopping. Initially, a previous batch of acceptable-quality spontaneously fermented dough was used to inoculate fresh dough at a level of 10%. The procedure was repeated every 24 hr at 30°C until a stable culture indicated by pH, titratable acidity, and viable microbial numbers was obtained. This was then used successfully to ferment dough within 24 hr at 30°C to the required level of acidification, a total titratable acidity value of 1.24%, calculated as lactic acid on a wet-weight basis, and a pH of 3.79 (42).

A starter culture containing strains of *L. fermentum*, *S. cerevisiae*, and *C. krusei* has also been developed for the production of fermented maize dough. Both in laboratory trials and at a commercial production site, the period of fermentation could be reduced from 48–72 hr to 24 hr. The organoloeptic qualities of the kenkey and *koko* prepared from doughs fermented with the starter for 48 hr were not significantly differnt from the traditional products. However, kenkey prepared from doughs fermented for 24 hr with starter culture were found to be unacceptable by the taste panel although similarly produced *koko* was acceptable (43–45).

IX. SEMI-INDUSTRIAL PRODUCTION OF KENKEY

At the Food Research Institute of the Council for Scientific and Industrial Research, Ghana, a pilot plant has been established for semi-industrial scale production of dehydrated fermented maize meal and kenkey using modern methods of food processing. A brief description of the plant with respect to plant layout, Good Manufacturing Practice, processing procedures, and an HACCP system (46) is given below.

A. Plant Layout

The objective of the layout was to permit an orderly flow of material through the plant during processing, give enough elbow room for the processing staff during processing and cleaning of equipment, and prevent cross-contamination of maize with fermented dough or the final product. The processing hall is divided into the main hall, the milling room, the fermentation room and the cooking section. This layout reduces noise pollution of the

plate mill by its seclusion in a milling room, the possibility of cross-contamination of fermenting dough by maize at the other stages of processing and excessive warming up of the processing hall by the partitioning of the cooking area. Fixed surfaces of the infrastructure that come into contact with the maize have an inert surface. A tap is installed at the top of each washing trough and steeping tank with outlets for draining at the base of each facility.

The design of all equipment fabricated for the plant has parts that are accessible for efficient cleaning. Materials used for fabrication of equipment are resistant to corrosion and easily cleaned. Equipment are located in the plant to provide ease and convenience of raw material and product handling, occupational safety of processing staff and prevention of microbial and physical contamination of maize, dough, or kenkey. The equipment is placed to permit adequate maintenance and cleaning and facilitate good hygienic practices and function in accordance with their intended use.

B. Maize Delivery, Storage, Cleaning, and Washing

Maize grains are purchased and delivered in jute sacks at the plant. Before purchase they are inspected for visible mold growth and extent of dryness. After weighing, the maize is cleaned in a mechanized dry cleaner. The cleaned grains are washed manually and rinsed in two large troughs to remove adhering sand and other water-soluble contaminating materials.

C. Steeping and Milling

The clean grains are steeped for 24 hr in concrete troughs. Because fermentation of the maize starts during steeping, the starter culture—made up of a strain of *L. fermentum* and a strain of *S. cerevisiae*—is suspended in the steep water before the grains are added. The strains originate from maize fermentations and they are added at a rate of 10^7 CFU/mL of steep water. The dry starter culture contains approximately 10^9 CFU/g.

The starter culture itself is produced in a 2 liter fermentor in the laboratory using a 10% slurry of malted maize flour as a substrate. The cells are harvested after cultivation by centrifugation and dehulled soybean flour is added in a ratio of 1:1 as a carrier. The mixture is dried in an oven at 42°C to a moisture content of 10%, and the flour is usually kept under refrigeration, though on shelf storage viability is still acceptable after a month storage at ambient temperature. After steeping, outlets at the base of the troughs are opened to drain the grains, and the grains are milled.

D. Fermentation

The milled maize is manually mixed with water into a stiff dough of moisture content of about 50 to 55% in plastic containers in about 30 kg portions. In some cases the starter culture is added to the water that is used to mix the moist flour into the dough. The plastic containers are placed on shelves in the fermentation room and left for 48 hr to continue fermentation till a pH of about 3.7 is achieved.

E. Production of Kenkey and Fermented Maize Meal Flour

The fermented dough is either used to produce kenkey or dehydrated into a shelf-stable flour that is marketed and used to produce *banku*, a stiff porridge made by directly cooking

fermented maize meal, sometimes mixed together with fermented cassava meal. The fermented flour is also used to make *koko*, a thin breakfast porridge. Kenkey is made as described earlier. For the production of the fermented maize flour, the dough is broken up and spread on shallow trays on trolleys, which are wheeled into walk-in mechanical dryers for dehydration of the dough (Fig. 4). Batch drying of the dough takes 6 to 10 hr, depending on the load. The dried dough is milled in a plate or hammer mill, sieved, and bagged in 1 kg sachets for sale.

F. Hazard Analysis Critical Control Point (HACCP) System for Kenkey Production

An HACCP system that is suitable for kenkey production at both traditional and upgraded commercial production sites has been developed (46).

Hazards associated with kenkey production and consumption are contamination with aflatoxins and other mycotoxins from maize grains and corn husks used for processing, survival and growth of spoilage and pathogenic microorganisms during steeping and fermentation, and various foreign materials and items. Maize sold on the open market is often contaminated with molds, including mycotoxin-producing species such as *Apergillus flavus* and *Aspergillus parasiticus*, which produce aflatoxins, and *Penicillium citrinum*, which produces citrinin. The occurrence of aflatoxins in maize and maize products in Ghana has been reported (47) and is a matter of public health concern.

Contamination of maize with molds is largely dependent on the moisture content of the grains and storage conditions. Field infestation of maize with molds may occur if there is intermittent rain during the period that mature maize is left to dry on the field before harvest. However, prompt harvesting of mature grains and mechanical drying to a moisture content of not more than 12% and efficient storage prevents mold growth.

The second hazard encountered in kenkey production is the risk of spoilage of steeped grains and fermented dough due to growth of spoilage microorganisms. Proliferation of such microorganisms in the products may result in an economic loss to the producer because the organisms produce strong offensive odors, which are not acceptable to consumers.

A third possible hazard in kenkey production is the survival and growth of pathogenic microorganisms even though the lactic acid fermentation of maize during kenkey has been shown to have antimicrobial effects against major bacterial food pathogens (24).

The prolonged cooking of kenkey for about 3 hr is drastic enough to kill off any surviving pathogenic bacteria and most of their toxins. Some mycotoxins produced by molds such as citrinin are also destroyed during kenkey boiling but aflatoxins are heat-stable and survive the process even though there is reduction in their total level (47).

The raw material, maize, constitutes the most important Critical Control Point (CCP) in kenkey manufacture because it is the point where mycotoxins can be introduced into the food operation. This CCP can be controlled by selecting suppliers capable of delivering maize that is not contaminated with aflatoxins and other mycotoxins. Such maize will not have any evidence of mold growth, be dry and crisp to bite, and have a clear color and all visual attributes of very good quality maize. Water used should be clean, colorless, and odorless.

Dry cleaning or sorting of maize is a CCP although it does not affect the microbiological safety of kenkey. However, is a very important step in production because it

greatly influences the organoleptic quality of the product. Maize purchased in sacks from the open market often contain a lot of debris such as broken cobs, soil, pieces of nylon thread used to knit the open end of the sacks, and other foreign material. Processors handle large quantities of maize, so they find it rather tedious to clean the maize by sifting and picking out unwanted materials manually. Maize cleaning is therefore often carried out inefficiently. Hazardous materials may also occur in the form of metal pieces broken off the grinding plates of the plate mill during milling.

During steeping, spoilage of maize grains may occur due to growth of spoilage microorganisms, which impart offensive odor to the maize. The growth and survival of the spoilage *microorganisms* is favored by higher pH values. A rapid drop in pH to acidic conditions as a result of the fermentation during steeping minimizes the occurrence of this hazard. This CCP can be monitored by pH measurement.

Fermentation is also a CCP. During fermentation spoilage of dough may occur if spoilage microorganisms are able to proliferate and pathogenic bacteria may survive. This is prevented by a rapid and pronounced drop in pH creating unfavorable conditions for growth of such microorganisms and production of antimicrobial compounds by lactic acid bacteria. This CCP can be monitored by pH measurement.

During packaging, contamination of the product with aflatoxins can occur if moldy maize husk or plantain leaves are used to wrap the balls. This CCP can be monitored by careful visual inspection of packaging materials for surface mold growth. The maize husks and plantain leaves should be washed just before they are used.

The prolonged cooking of kenkey kills any surviving spoilage and pathogenic microorganism, except sporeformers (e.g., *Bacillus* spp), which are not able to develop at the low pH, and as mentioned earlier, some mycotoxins are destroyed or reduced in quantity (47). Cooking also prolongs the shelf life of the fermented dough by killing off the microflora. This CCP can be controlled by ensuring that kenkey is always cooked for a minimum of 3 hr on an open fire or 1 hr in a pressure cooker.

The critical limits for each of the CCP, which ensures that kenkey produced will satisfy the intended toxicological, microbiological and sensory quality, can be summarized as follows: Maize to be processed should be undamaged, have a moisture content of about 12%, and show no visible mold growth. Water used for washing, steeping, or dough kneading should be colorless, odorless, and neutral in taste. The pH of steepwater at the end of steeping should be below 4.2 within 24 hr and steeped maize should not have an offensive smell. The pH of fermented dough should be below 3.9 within 48 hr, with a lactic acid content of about 1.4%, acetic acid content of 0.18–0.23%, and a volatile to nonvolatile acid ratio of about 0.16. The fermented dough should not have an offensive smell and maize husks and plantain leaves used for packaging should show no visible mold growth.

The HACCP system can only be effective if Good Manufacturing Practices (GMP) are adhered to. Good housekeeping, good personal hygiene of staff, cleaning of equipment and processing rooms are general requirements for safe and otherwise acceptable kenkey production. Noncompliance to such codes of conduct and practices will lead to contamination of materials during processing, thus posing a hazard. For example, the contamination of processing materials will occur during milling, dough preparation, and molding of *aflata* dough mixture into balls if good personal hygiene is not observed during these operations. Potable water, clean equipment and containers, and so forth should be used at all times. Training of workers in hygienic conduct and understanding of the basic principles of food hygiene is very important. Efficient GMP should be monitored by regular inspection.

X. CONCLUDING REMARKS

Kenkey is one of the best examples of traditional African foods, which through history have played a significant role in food safety as well as food security. The underlying fermentation process has provided foods of highly appreciated properties and represented an art of food preparation and preservation with substantial socioeconomic impact in West African societies. The raw materials used are all indigenous and the sustainability of this type of food processing is unique. The industrialization of the global agribusiness system has strongly concentrated opportunities for adding value to the process end of the chain. Development of traditional food fermentations, like processing of kenkey, into scientifically based and controlled industrial operations is a way for Africans to take their share of this global development. To continue to be producers of plant raw materials appears not to be sustainable.

Through recent intensive research activities, the microbiology of kenkey is now very well understood. Effective starter cultures have been identified and shown to improve maize fermentations leading to the preferred characteristics of kenkey. The cultures appear to have desired functional properties and they have been defined by detailed pheno- and genotyping, which can also provide a proper background of patenting of cultures and protecting intellectual rights.

It has been demonstrated how traditional production sites can be upgraded to meet the requirements of recognized guidelines of GMP. Local producers, consultants, and organizations have been trained in the use of the HACCP concept for management of food safety in maize processing. Kenkey flour with an extended shelf life has been developed. The final step of development, to achieve a product that is acceptable and attractive to European consumers, seems no longer to be out of reach. The next move is directed toward development of governmental agencies for independent and qualified verification and validation of the safety and quality of kenkey as well as hygienic conduct and understanding of the basic principles of food hygiene in the complete chain from growing and handling of maize through processing of kenkey.

The important role of microbiology in African foods has been demonstrated by the biodiversity and promising properties of the dominating microorganisms in the many different fermented foods, including kenkey, in Africa. It justifies that microbiology be made a high-priority research area in Africa to support a development in biotechnology. Potential biotechnological activities could include, for example, production and sales of starter cultures, probiotic cultures, cultures for biopreservation, and bioactive substances for various purposes.

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Fermented Liquid Milk Products

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I. INTRODUCTION

Fermented milk products have been an important part of the human diet for thousands of years. These products are believed to have originated in the Middle East region as a means of extending the shelf life of milk, and over time they have permeated around the world. They now form an important industry in most countries of the world (1), and consumption in many countries is considerable (2), especially in Europe (Table 1). Scandinavian countries and Russia are among the world's largest consumers of fermented milks (1).

In the early years, the objective in fermented milks manufacture was primarily that of production of acid (usually lactic) to enable extended storage of milk in the absence of refrigeration. Now, consistency in fermentation and production of selected specific flavors along with a clean acid flavor are the norm. With the proper selection and propagation of cultures, it is possible to produce numerous types of cultured products with a wide range of flavor and texture characteristics. In addition, these products offer various functional and nutritional characteristics for ingredient applications and therapeutic value for the maintenance of proper intestinal microbial ecology. The therapeutic properties of fermented milks have been recognized for many years (3). Many of these properties are generally not available in the original milk from which they are produced. Fermented milk products have thus added much to the diversity of the diet. While the commercial technology for the production of these products has advanced significantly in the realms of cultures, production techniques and packaging, many types of cultured products are routinely prepared on a small scale in the home because of the relative ease of manufacture.

A selection of fermented milks from around the world is listed in Table 2. A comprehensive list can be found in the Encyclopedia of Fermented Fresh Milk Products (4) and some are described elsewhere (5,6). The primary focus of this chapter is on the discussion of acidophilus milk, kefir, and koumiss.

II. ACIDOPHILUS MILK

Acidophilus milk is produced mainly by the fermentation of milk with *Lactobacillus acidophilus* and it can have up to 2% lactic acid (1). The manufacture of acidophilus milk is outlined in Fig. 1. With DNA-DNA homology studies, the acidophilus group of bacteria

Table 1 Consumption of Fermented Milks Around the World, 1998

Country	Per capita (kg)	Country	Per capita (kg)
Netherlands	45.0	Czech Republic	10.0
Finland	38.8	Portugal	9.8 (1997)
Sweden	30.0	Hungary	9.4
Denmark	27.3	Poland	7.4
France	26.9	Slovakia	7.4
Iceland	25.3	USA	7.1 (1997)
Germany	25.0	Australia	6.4
Israel	24.8	Argentina	6.0
Norway	19.3	Canada	3.6
Bulgaria	15.6	Ukraine	3.4
Austria	14.7	South Africa	3.1
Spain	14.5	China	0.2

Source: Ref. 2.

has been classified into six major species: *Lactobacillus acidophilus* (A1), *Lactobacillus crispatus* (A2), *Lactobacillus amylovarus* (A3), *Lactobacillus gallinarum* (A4), *Lactobacillus gasseri* (B1), and *Lactobacillus johnsonii* (B2) (7). It is possible therefore, that acidophilus milks manufactured prior to this classification may have actually contained one or more of the above species. Currently, *Lactobacillus acidophilus* and *Lactobacillus johnsonii* are used in the manufacture of fermented milks commercially.

Therapeutic properties of *Lactobacillus acidophilus* have been recognized for long (8) and it is believed that this organism is able to implant in the large intestine and survive (9,10). Consequently, acidophilus milk is found in health food stores and is used as a therapeutic product. A major characteristic that limits its consumption is poor palatability due to its extremely high acid content. Furthermore, the sterilization treatment applied to the milk also imparts a strong cooked flavor to the product. Most strains of *Lactobacillus acidophilus* are not able to survive the high acid conditions created by their growth in milk so maintenance of high numbers in products is difficult. Various alternative products have therefore been developed that have better flavor qualities but supposedly have therapeutic properties similar to acidophilus milk because of the presence of large population of *Lactobacillus*

Table 2 Examples of Fermented Milks

Name	Characteristics
Acidophilus milk	Medium acid
Bulgarian buttermilk	High acid
Cultured buttermilk	Low acid
Kefir	Acid and alcohol
Koumiss (Kumys)	Acid and alcohol
Lassi	Medium acid (with added salt or sugar)
Langfil	Medium acid, ropy
Mala	Medium acid with added fruits
Viili	Medium acid, mold, ropy

Source: Refs. 1 and 4.

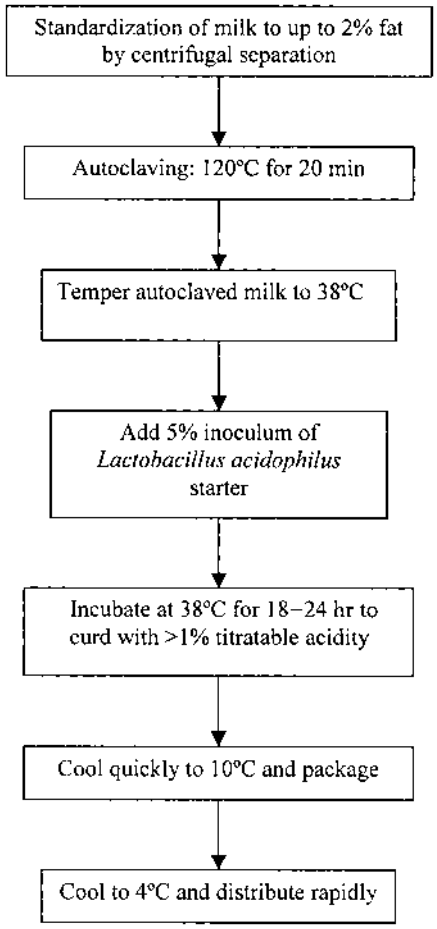

Figure 1 The manufacture of acidophilus milk.

acidophilus. Among these are sweet acidophilus milk (11), also marketed as AB milk in the USA; BRA in Sweden (1); Actimel (Dannon company), which contains *Lactobacillus casei*; and LC1 of the Nestle company in Switzerland. The latter product contains *Lactobacillus johnsonii*.

As implied by the name, some of these alternative liquid products are not fermented, hence the term "sweet." Early versions of sweet acidophilus milk contained only *Lactobacillus acidophilus* but now also include one or more strains of bifidobacteria. The strain used for such milk generally is *Lactobacillus acidophilus* NCFM, which is a member of the *Lactobacillus acidophilus* A1 group (12). The BRA milk of Sweden has viable bifidobacteria, *Lactobacillus acidophilus*, and *Lactobacillus reuteri*. The latter produces an antibiotic (reuterin) that inhibits the growth of undesirable bacteria in the intestinal tract. In the manufacture of these acidophilus beverages, concentrated cell masses of the above selected bacterial strains are added to pasteurized, cooled, low fat milk, packaged and stored at 4°C until consumed. The cell count of the added bacteria is approximately 5 million per milliliter of milk. There is no fermentation, hence no acid is produced but the consumer ingests large amounts of live lactobacilli and/or bifidobacteria cells that are subsequently implanted in

the intestinal tract. The shelf life of such milks is limited to 2 weeks under refrigeration because of the presence of live lactic acid–producing bacteria. Extended storage under refrigeration or exposure to higher temperatures during storage will initiate the growth of the added cultures and rapid production of acid, followed by curdling of milk, which would be regarded by consumers as spoilage even though the curdling is caused by lactic acid bacteria.

In Finland a liquid product is manufactured by fermenting demineralized, lactose-hydrolyzed whey concentrates or concentrated whey with *Lactobacillus* strain GG (1). This strain, named after Goldin and Gorbach, the scientists who discovered the organism, is isolated from the human intestinal tract and is believed to possess therapeutic properties, especially those pertaining to gastrointestinal disorders. This product is produced with or without any added fruit flavors and has a pleasant acid flavor.

III. KEFIR

Kefir has a long history in Russia, where it originated, but is now manufactured and consumed in several other parts of the world as well (1,13), though the per capita consumption remains high in Russia at 4 to 5 kilograms. The name kefir is derived from the Turkish term *kef* or from kefy in the Caucusus region implying pleasant taste (4). Kefir is a viscous pourable liquid, with a smooth, slightly foamy body and whitish color. It is yeasty, acidic, mildly alcoholic, refreshing, and slightly effervescent. Kefir is generally made from cow's milk but in the early years it was also made from goat's and sheep's milk in leather sacks. It perhaps still is in some rural areas. Industrial manufacture of kefir began in Russia in 1930 (14).

Like many other milk products, kefir ranges in fat content from nonfat to 3.5% but it is in many ways a unique product in that it entails acid and alcohol fermentation. Alcohol fermentation is the result of the addition of yeasts in the form of kefir grains. Because of the multiple fermentation process, the resulting product possesses flavor that is characterized by a balance of lactic acid (0.8%), diacetyl, acetaldehyde, and ethanol (1%). Diacetyl and acetaldehyde in a 3:1 ratio provide optimum flavor. The complex flavor is rounded out by fizz from carbon dioxide that is also produced during fermentation.

Kefir is manufactured using one of two main procedures. In the traditional process, kefir grains are employed for fermentation. Another method involves the use of lyophilized concentrates of cultures. In the latter method, kefir grains are not used.

A. Manufacture of Kefir with Grains

Kefir may be manufactured with grains in one of two ways (1). In the traditional system, kefir grains are added to cow's milk that has been heated to 85°C for 30 min and cooled to 22°C. Incubation is for 24 hr at 22°C with occasional stirring. The grains are sieved when they rise to the surface due to carbon dioxide production, and ripening for alcohol production continues at 10–15°C for 24 hr.

In another process, a kefir starter is first obtained from the grains for fermentation. Here, freeze-dried kefir grains are rehydrated in a sterilized 0.9% sodium chloride solution at 20°C for 5 hr. The grains are then washed with sterile water and added in a 1:30 ratio to skim milk that has been heated to 95°C for 30 min and cooled to 25°C. After incubation for a day, the grains are sieved and the process of fermentation in skim milk is repeated two more times. After the final fermentation, the grains are sieved and the skim milk without the grains

is then added as the kefir culture at the rate of 5% to milk that has been heated to 85°C for 30 min and adjusted to 22°C. Fermentation ensues for 12 hr (pH 4.5 to 4.6) followed by ripening for 1 to 3 days at 8 to 10°C. During ripening, yeast fermentation occurs.

The ratio of kefir grains to milk affects the microflora of the starter. If a relatively large proportion of grains are added to milk (e.g., ratio of 1:10), the proportion of lactococci and yeasts is lower than when the proportion is smaller (1:30 to 1:50) because of the rapid accumulation of lactic acid. The optimum development of all groups of organisms in the grains takes place at a ratio of 1:50 (14).

B. Manufacture of Kefir from Concentrated Culture

Kefir may also be produced with a concentrated culture that is prepared from isolates obtained from kefir grains (1,15). In the actual manufacturing process, no grains are used, as the organisms have already been isolated as a concentrated culture. This culture consists of 75% homofermentative lactococci, 24% citric acid–fermenting lactococci, 0.5% lactobacilli, and 0.1% *Candida kefir* yeasts. A bulk starter made from this concentrated culture is added to heated milk (94°C, 5 min, cooled to 22°C) at the rate of 1%. Incubation is for 18–22 hr until pH is 4.5 to 4.6.

C. Kefir Grains

Kefir grains are the most important components of kefir manufacture (Fig. 2). The concept of kefir grains is believed to have originated in the accidental discovery of kefir many hundreds of years ago in the Caucasus region. These grains vary in size from a wheat grain to the size of a walnut. They are of whitish to yellowish color and gelatinous and irregular in

Figure 2 Kefir grains. (Courtesy F. V. Kosikowski LLC.)

shape and with a rough surface. The dominant microflora of kefir grains are listed in Table 3 and consist of mesophilic, homofermentative, and heterofermentative lactococci, heterofermentative lactobacilli, lactose-fermenting and lactose-nonfermenting yeasts, and acetic acid bacteria. The microbial ecology within the grains depends on the origin and method of cultivation of the grains (4,16–18). In one study, 120 strains of lactobacilli were isolated from kefir grains. The predominant strain was *Lactobacillus kefirogranum* (19). These groups of bacteria when purified do not grow at all or grow very slowly in milk. In fact, it has been demonstrated that manufacture of good-quality kefir by using isolated bacteria is difficult (20). Thus, the symbiosis within kefir grains is critical in optimal growth for kefir production. Koroleva has described such symbiosis (14): proteolysis and vitamin production by yeasts and acetic acid bacteria stimulates lactic acid bacteria, which in turn utilize lactose to promote growth of lactose-nonfermenting yeasts. The metabolites of growth of lactic acid bacteria also check the growth and alcohol production by yeasts.

Newer strains from kefir grains such as *Lactobacillus kefiranofaciens* (21) and *Saccharomyces turicensis* (22) have been isolated. It is conceivable that as new microbiological techniques become available, newer strains will be identified and the microbial ecology of kefir grains will be better understood.

Kefir grains may be contaminated with coliforms, bacilli, micrococci, and mold, which will rapidly spoil the product manufactured with such grains. According to Koroleva, a good quality kefir culture prepared in skim milk should contain 10^8–10^9 lactococci, 10^7–10^8 leuconostocs, 10^5 thermophilic lactobacilli, 10^2–10^3 mesophilic lactobacilli, 10^5–10^6 yeasts, and 10^5–10^6 acetic acid bacteria per milliliter (23).

Kefir grains consist of approximately 24% polysaccharide called kefiran, which consists of glucose and galactose in equal proportion and is not easily attacked by enzymes. Various lactobacilli such as *Lactobacillus kefiranofaciens* (24) and others (25,26) that are present in the grains produce these polysaccharides. The polysaccharide content of kefir is approximately 0.2–0.7% and provides a slightly ropy texture to the final product.

Table 3 The Microflora of Kefir Grains

	Species
Yeasts	*Candida kefir*
	Candida pseudotropicalis
	Kluyveromyces marxianus subsp. *marxianus*
	Saccharomyces kefir
	Saccharomyces turicensis
	Torula spp.
	Other yeasts
Bacteria	*Acetobacter aceti*
	Lactobacillus casei
	Lactobacillus helveticus
	Lactobacillus kefir
	Lactobacillus kefirogranum
	Lactobacillus kefironafaciens
	Lactococcus lactis subsp. *lactis*
	Leuconostoc mesenteroides subsp. *dextranicum*
	Leuconostoc mesenteroides subsp. *cremoris*

Kefir grains are an excellent example of the coexistence of yeasts and bacteria. When kefir grains are soaked in milk, they swell and turn white and fermentation proceeds. During this process the grains also increase in numbers, possibly by splitting into smaller ones. Viable microbes from the grains are also shed into the milk during this process. The grains can be propagated and recovered in this manner and used in subsequent kefir production but it has not been possible to synthesize kefir grains.

Newly purchased kefir grains become fully active after two or three propagations (1). Kefir grains when left wet have a limited shelf life. When stored at 4°C they lose activity within 8 to 10 days if not used. Dried grains are active for 12 to 18 months of storage. Kefir grains if properly used and maintained can be reused many times. Grains that are recovered in a strainer should be washed with sterile water and can be stored wet in cold sterile water for reuse within a few days. Excessive washing can alter the microflora of the grains and hence the quality of the final product. They can be dried at room temperature for 36–48 hr and stored in a cool and dry place until reused (1) or be maintained in frozen state (−20°C) for extended storage (27).

In place of kefir grains, freeze-dried kefir microflora have been suggested for kefir manufacture for uniformity in quality (15). This was accomplished by cultivating kefir bacteria and yeast isolated from grains under controlled pH and incubation conditions. Bacterial concentrates obtained by centrifugation were freeze-dried with protective agents with survival rates of up to 70%. Including 20% sucrose and starch solutions enhanced survival rates of yeasts during freeze-drying.

D. Application of New Technologies

Kefir has gained popularity in may parts of the world outside its location of origin because of its refreshing taste and potential therapeutic value. The product has therefore continued to evolve and manufacturing procedures have been modified to produce products that meet consumer preferences and also improve the efficiency of manufacture.

Acid and alcohol fermentation can continue in finished kefir during storage because of the relatively high residual lactose content and the presence of yeasts. The result of this is extremely strong and undesirable products. Methods have been developed to control such post-production fermentation. In one such method (28), kefir is manufactured with selected lactic acid bacteria and yeasts. The selected lactic acid bacteria, which include *Streptococcus fecalis*, *Lactobacillus casei*, *Lactobacillus lactis*, *Lactobacillus fermenti*, and *Lactobacillus yogurtii*, are able to hydrolyze galactose and prevent the buildup of galactose. Yeasts strains that do not hydrolyze lactose and galactose are used (e.g., *Saccharomyces bisporus*, *Saccharomyces mellis*). Calculated amounts of sugars fermented by these yeasts such as glucose and fructose are added such that all sugar is utilized by the time the required amount of alcohol has been produced. During storage, therefore, there is no more alcohol production.

Kefir with enhanced nutritional value may be produced by the application of ultrafiltration (29). In this process, milk is ultrafiltered to raise the solids content by 0.5–4%. This increases the protein content and reduces the lactose content of the milk and hence kefir. After high heat treatment, this milk is fermented to a pH of 4.5 to 4.7 and packaged. This product is especially designed for patients who rely heavily on kefir for therapeutic purposes but do not have other significant sources of protein. It has been recommended that for manufacturing kefir, skim milk should be ultrafiltered to no more than 1.8-fold in order to retain optimum texture quality (30). The application of ultrafiltration to increase the protein content by 0.2 to 0.3% is also useful for improving the consistency of low-fat kefir (31).

Kefir products with added value have also been developed, such as kefir fortified with a patented colostrum product for enhancing the immune system (32). Kefir products with prebiotics such as fructooligosaccharides (33) and probiotics such as bifidobacteria (34) have also been developed.

IV. KOUMISS (*KUMYS*)

Koumiss is a frothy beverage with a grayish-white color, a refreshing taste, and a characteristic aroma (1). The name koumiss is derived from the Kumanes tribe of the central Asian steppes, where it is believed to have originated many hundreds of years ago and was a common drink of the Tatar and Mongol tribes. Traditionally, this product was obtained by fermentation in a vessel made of horse-skin (35), but now it is made on a large scale in some parts of the world from mare's milk. It is an important product of Russia and Mongolia, where horses have been maintained especially for the production of this product. A distinctive feature of koumiss is that it, like kefir, is also produced by acid-alcohol fermentation. Koumiss is regarded as a product of high therapeutic value and a thirst-quenching beverage of high nutritional value in Russia.

Vladimir, Soviet, and Russian heavy draft horse breeds are used for koumiss production in Russia and they yield 10 to 20 liters of milk per day (36). Milking occurs every 2 to 3 hr because of the low milk-holding capacity of the udder (37). Mare's milk resembles human milk more than it does cow's milk. On average, it has 1.9% fat, 1.3% casein, 1.2% whey proteins, 6.2% lactose, and 0.5% ash (38), which gives it a lower viscosity than cow's milk. There is no visible curd when the milk is set and there is no wheying off. Protein characterization studies of mare's milk has shown that the isolectric point of casein is 4.2 compared to 4.6 for cow's milk (39).

Although koumiss is traditionally made from mare's milk, limited availability of such milk has led to the development of procedures for its manufacture from cow's milk as well. There is however, a concerted effort in Russia to increase the production of mare's milk for koumiss production via improvements in horse breeding and management techniques (40–42).

There are several types of koumiss available, depending on the amount of acid and alcohol (Table 4). The manufacture of koumiss therefore requires controlled lactic acid using *Lactobacillus delbrueckii* subsp. *bulgaricus* and alcohol fermentation using yeasts such as *Torula* spp. and others. In addition to acid and alcohol, carbon dioxide is also produced to impart fizziness to the final product.

Various regional products similar to koumiss are also made in central Asia on a relatively small scale: Examples include Chal, which is manufactured from camel's milk, Kuban, and Kurunga (4).

Table 4 Types of Koumiss

	Lactic acid, %	Alcohol, %
Weak	0.7	1.0
Ordinary	1.1	1.8
Strong	1.8	2.5

A. Manufacture of Koumiss

1. Mare's Milk

Koumiss can be manufactured using a previous batch as bulk starter or by preparing a bulk culture from pure strains. For preparing a bulk starter, yeast and lactic cultures are propagated separately (1). Two liters of nonfat cow's milk that has been heated to 70°C for 30 min and cooled to 30°C is inoculated with *Torula* spp. yeast. Incubation is for 15 hr at 30°C. A liter of similarly treated milk is inoculated with a lactic culture (*Lactobacillus delbrueckii* subsp. *bulgaricus*) and incubated at 37°C for 7 hr. The two separately fermented milks are mixed with a liter of mare's milk and incubated at 28°C. Additional mare's milk is added until about day 4 when the acidity is 1.4%. This mixture is then used as bulk starter for the production of koumiss as outlined in Fig. 3. Aeration during incubation is important to allow the yeasts to grow. The three categories of acid-alcohol in koumiss cited in Table 4 can be achieved by adopting short or long ripening periods as follows: 1 day for weak, 2 days for ordinary, and 3 days for strong. Obviously, koumiss should be consumed quickly after production as acid and alcohol production may continue during storage and the product will become stronger.

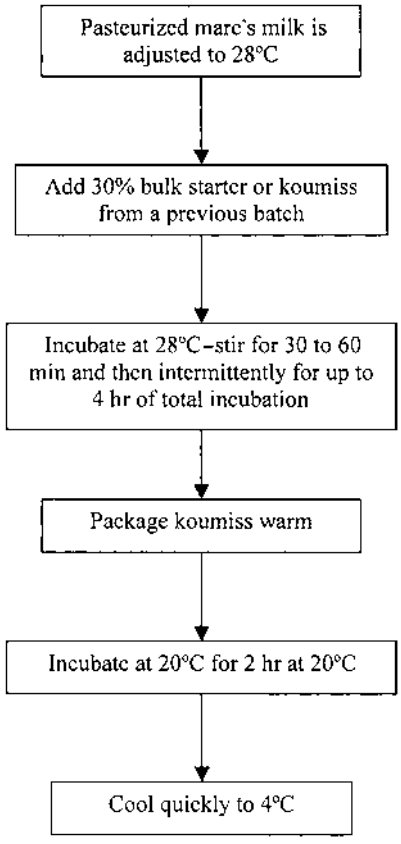

Figure 3 Manufacture of koumiss from mare's milk.

2. Cow's Milk

Koumiss that is similar in sensory and therapeutic qualities to mare's milk can be manufactured from cow's milk if appropriate adjustments are made for composition (43). As stated above, mare's milk has a significantly lower protein content and higher lactose content than cow's milk and the proportion of whey proteins is higher relative to casein in mare's milk. In one procedure utilizing cow's milk (1), 2.5% beet sugar is added to skim milk and pasteurized and a 10% culture consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Saccharomyces lactis* is added. The mixture is incubated at 27°C until curd forms and acidity is 0.8%. It is then held at 17°C with aeration via stirring until acidity is 0.9%. Aeration permits the growth of yeasts. The product is then packaged and ripened for a further 2 hr at 17°C before cooling to a storage temperature of 4°C.

In another procedure (23), cow's milk is first standardized as follows: 34.6 parts whole milk, 0.8 parts skim milk, and 64.6 parts of cheese whey are mixed. Starter similar to the one described above for cow's milk is added to the mixture at the rate of 20%. Ascorbic acid is also added at the rate of 0.2 g per kilogram. Standardization lowers the casein content and raises the whey protein and lactose contents. Ascorbic acid is added as a supplement to match the vitamin C content of mare's milk. The mixture is incubated at 28–30°C for 3 to 4 hr under continuous stirring. When the titratable acidity is approximately 0.7%, it is cooled to 16–18°C and stirred for 1 to 2 hr. The product is then packaged and ripened at 6–8°C for 1–3 days.

A Swiss process (44) employs the ultrafiltration of whey prior to standardization of cow's milk to simulate the composition of mare's milk. The lactose in the mix is partly hydrolyzed by beta-galactosidase to improve yeast fermentation. The sensory quality and composition of the resulting koumiss were similar to that made from mare's milk.

B. Starters

In the early years, koumiss from a previous batch was used as starter but purified starters have since become available (14). Such purified starters may be in the form of concentrates

Table 5 Microflora of Koumiss

	Species
Yeasts	*Candida kefir*
	Candida buensis
	Kluyveromyces marxianus subsp. *lactis*
	Saccharomyces unisporus
	Saccharomyces cartilaginosus
	Other yeasts
Bacteria	*Lactobacillus causasicum*
	Lactobacillus curvata
	Lactobacillus delbrueckii subsp. *bulgaricus*
	Lactobacillus paracasei subsp. *paracasei*
	Lactobacillus paracasei subsp. *tolerans*
	Lactobacillus rhamnosus

Source: Refs. 14, 46 and 47.

and include *Lactobacillus bulgaricus* and *Saccharomyces lactis* (45). The microflora of commercial koumiss is a reflection of the microflora of the starter. Various strains of lactic acid bacteria and yeasts have been isolated from commercial koumiss. One study, for example, isolated 43 strains of lactic acid bacteria and 20 strains of lactose-fermenting yeasts (46) and another isolated 417 yeasts cultures from 94 samples of koumiss (47). Lactose-fermenting as well as lactose-nonfermenting yeasts were isolated (Table 5).

V. THERAPEUTIC PROPERTIES OF LIQUID FERMENTED MILK PRODUCTS

Medicinal value of liquid fermented milk products has been suggested for hundreds of years, and various studies have implied that individuals for whom fermented milks such as acidophilus milk, Bulgarian buttermilk, and kefir is a part of the diet as in some parts of Russia, live long lives (1). This value goes beyond the accepted high nutritional quality of unfermented milk. The fermentation process apparently adds therapeutic qualities to milk that are not found in the original milk. These properties are imparted by the cultures that have the ability to survive product manufacturing and storage procedures and the harsh environment in the gastric system and establish themselves in the intestinal tract. Through their activities in this environment they are able to perform functions that are of benefit to the consumer. Also, some metabolic products of their growth during the fermentation process add therapeutic value to the finished fermented product. For example, a pasteurized Japanese product (Ameal S) is produced by the fermentation of a milk-based medium with *Saccharomyces cereviseae* and *Lactobacillus helveticus*. The fermentation process produces two tripeptides, valine-proline-proline, and isoleucine-proline-proline, which have the ability to reduce blood pressure. In this product, live bacteria do not exist and are not important but the products of their growth are (48).

Much has been written about studies on the therapeutic value of fermented milks involving in vitro, animal, and human studies. Various benefits have been suggested, including the reduction in risk of gastrointestinal illnesses such as diarrhea, improved lactose digestion, enhancement of the immune function, decreasing *Helicobacter pylori* infection, reduction of cholesterol and certain allergies, anticarcinogenic action and so forth (49,50). As a result of this, commercial fermented milks with health claims have been developed in some countries and in Japan the Ministry for Welfare developed FOSHU (Foods for Specified Health Use) regulations in 1993 for such products (51).

Lactobacilli such as the types used in the manufacture of some liquid fermented milks described above have been linked to various therapeutic properties (12). *Lactobacillus acidophilus*, *casei*, and *reuteri* are believed to control the growth of undesirable microorganisms such as *Escherichia coli* and *Salmonella* spp. in the intestinal tract (50). A large amount of acid produced by these organisms is one method of such control, as is competitive exclusion, but these bacteria are also able to produce bacteriocins that are antagonistic against undesirable organisms. *Lactobacillus acidophilus* LB (52), *Lactobacillus johnsonii* LA1, and *Lactobacillus casei* YIT9209 (53) produce antimicrobial substances against *Helicobacter pylori*, which is a human gastric pathogen. The antibiotic reuterin produced by *Lactobacillus reuteri* is effective against a wide range of organisms including pathogens (50). Control of undesirable microorganisms that produce carcinogenic metabolites in the intestines may also result in anticarcinogenic effects by limiting the production of such metabolites (50).

Some lactic acid bacteria have been shown to stimulate immune functions by activating macrophages, increasing the levels of cytokines, and increasing immunoglobulin levels (especially those of IgA). Organisms identified in such functions include *Lactobacillus johnsonii* LA1, *Lactobacillus* GG, and *Lactobacillus casei* Shirota, among others (48).

The improvement of lactose digestion, for which *Lactobacillus acidophilus* has particularly been identified, is useful for individuals who are not able to digest lactose. *Lactobacillus acidophilus*, unlike yogurt bacteria, is able to survive and grow in the intestinal tract. Here the increased permeability of the bacterial cells allows for the permeation of lactose and subsequent hydrolysis by beta-galactosidase (50).

A reduction in serum cholesterol has also been linked to certain lactobacilli including acidophilus, johnsonii, and casei (50,54). This activity is believed to occur by the assimilation of cholesterol or via deconjugation of bile acids in the small intestine and subsequent excretion from the body. Bile acids are precursors for cholesterol; hence their removal from the body helps in reduction of cholesterol.

The therapeutic properties of traditional kefir have been identified as an increase in the excretion of urea and some nitrogen metabolism products (14) and the treatment of atherosclerosis, allergic disease, and gastrointestinal disorders (55). The polysaccharides are able to decrease the cholesterol content of blood and bind toxins. Anticancer effects of kefir have also been suggested (14). The inclusion of pre- and probiotics, as stated earlier, and colostrum products have expanded the therapeutic value of kefir.

Koumiss made from mare's milk has been used widely in Russia for many years for the treatment of tuberculosis (56,57), and chronic enteritis and gastrointestinal disorders (58). The first hospital specializing in koumiss therapy was established in 1858 in Samara, Russia. The intestinal microflora is apparently normalized with koumiss therapy and partly because of the intense yeast fermentation, the vitamin B_{12} absorption and levels in blood are raised (59). The dose for adults is 1.5 L per day and 0.4 to 0.8 L for children (1). Koumiss is readily digested and assimilated because of its lower casein content and a larger proportion of peptones and free fatty acids that are generated during fermentation.

Many of these therapeutic properties of fermented milks are strain specific—not all strains have all of the above characteristics. In the manufacture of fermented milk, therefore, it is important to use specific strains if health claims are made (60–62).

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Partially Fermented Tea

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I. INTRODUCTION

It is generally accepted that tea is the most popular beverage besides water in the world. It has its own characteristic aroma and taste. In recent years, numerous research reports have proved its effects on health maintenance. Therefore, tea is popular worldwide.

Tea is made from the newly emerged tender leaves of the tea tree [*Camellia sinensis* (L.) O. Kuntz]. The tea tree is a perennial evergreen. Tea taxonomy is still a challenge today, not having received the attention it deserves, possibly because of the complexities involved (1). According to current taxonomy, tea trees belong to the *Camellia* genus of the Theaceae. It is a different species than the ornamental *Camellia* plants in gardens but belongs to the same genus. Within the *Camellia* genus, there are at least 90 or more species, and *Camellia sinensis* (L.) O. Kuntz is the only species that is widely made into a beverage. *Camellia sinensis* (L.) O. Kuntz has two big groups: the small-leaved *var. sinensis* and the large-leaved *var. assamica* that differ significantly in their growth habits, branching size, shape, texture and pose of leaf, as well as inherent yield and other characteristics. However, these two are still the main cultivated species processed into various tea beverages.

Theoretically, either large-leaved *var. assamica* or small-leaved *var. sinensis* can be processed into tea with various degrees of fermentation. However, because of considerations of flavor and quality, each variety (even cultivar) has its own suitability for processing into its own kinds of tea. In general, the large-leaved species is more suitable for making fully (completely) fermented tea, and the small-leaved species is used mainly for the making of unfermented or partially fermented teas.

Partially fermented tea is characteristic of China. China is probably the only country in the world that produces this kind of tea extensively. The world production of tea is about 2,900,000 metric tons per year. Black tea is the major tea, accounting for over 80% of total production; green tea is second, about 15% of the total. Because the productions of white tea, chingcha (Oolong tea), yellow tea, dark green tea, and scented tea are comparatively

minor, they are usually grouped into the "others" category. There is no accurate figure on the production of partially fermented tea or its percentage in world tea production. However, it is clear that the actual amount of partially fermented tea is minimal in the world tea market. The rough estimate is less than 100,000 metric tons. However, partially fermented tea has several distinct characteristics among all the teas: (a) its origin from a later date; (2) large deviation in processing procedures; (c) large variety of different kinds; (d) special/characteristic aroma; (e) unit price much higher than other teas; and (f) localized production and consumption. Therefore, it warrants understanding of its processing steps and flavor characteristics.

Besides, because of the popularity of Oolong tea in Japan in the past 20 years, high-class Oolong tea enjoys a stable high price in Taiwan. This has led some tea merchants to invest on the production of Oolong tea in countries such as Vietnam and Indonesia. This is a trend worth noticing.

II. CLASSIFICATION OF TEA

There are many kinds of tea. In general, tea can be classified, based on the degree of fermentation, into four big groups (Fig. 1). These are unfermented tea, partially fermented tea, fully (completely) fermented tea, and post-fermented tea.

The so-called fermentation in tea processing is, in fact, a misnomer. In the beginning, tea was considered to undergo fermentation like that of other kinds of fermented foods and beverages, with involvement of microorganisms. Later, it was proved that microorganisms

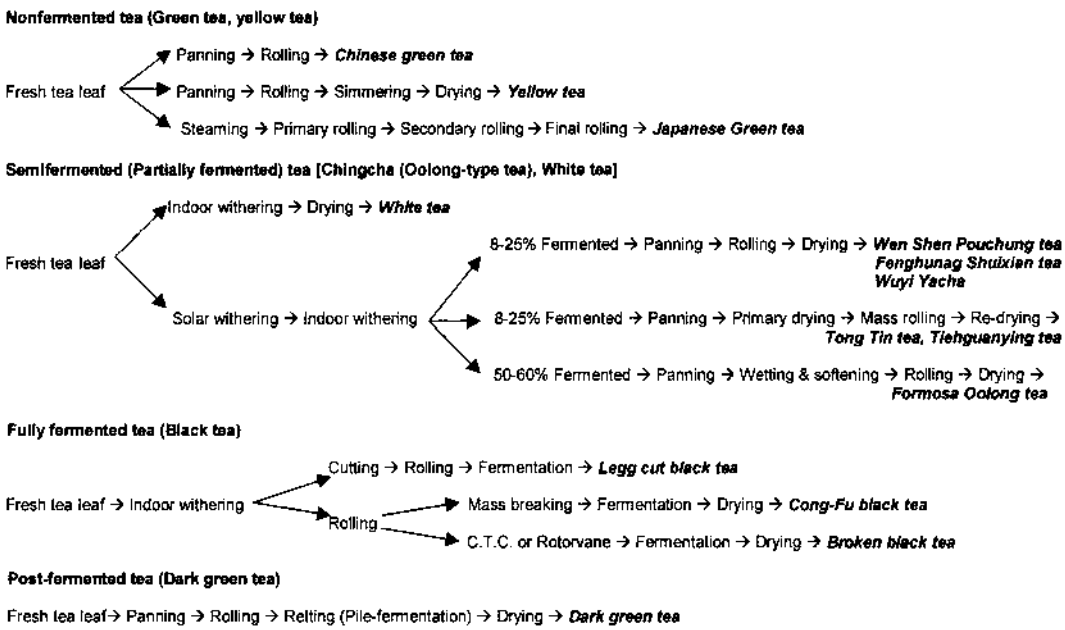

Figure 1 Kinds of tea and production process.

were not involved in this so-called fermentation process but that the process was the result of enzymatic oxidation induced by the tea leaf itself. However, fermentation has been wrongly used for a long time that it cannot be corrected. The term is still being used, and it is even the basis for classification of tea!

The most important reaction in tea fermentation is the oxidative chemical reaction induced by the polyphenol oxidase on the polyphenols in tea leaves. This subsequently causes the formation of different colors, aromas, and tastes in tea with various degrees of fermentation. In the manufacture of unfermented tea, the tea leaves generally are exposed to high temperature for the blanching process to inactivate the enzymes, thus no oxidation follows. In partially fermented tea, the fermentation process generally involves a withering or still shaking step to induce a slight oxidation in the tea leaf, followed by blanching to terminate the process. In the manufacture of fully (or completely) fermented tea, the tea leaves are withered, rolled, and fermented thoroughly, followed by drying to complete the process. In the manufacture of post-fermented tea, the first steps are the same as in the manufacture of unfermented tea, followed by pile fermentation to have microbiologically induced auto-oxidation and non-enzymatic auto-oxidation to complete the process.

In Mainland China, tea is classified into six basic groups [green tea, yellow tea, chingcha (Oolong tea), white tea, black tea and dark green tea] based on their different manufacturing processes and quality (2). This kind of classification can cover all kinds of tea, and at the same time demonstrates the differences in quality as well as the processing steps. It is now being accepted as a more systematic way of classification.

Partially fermented tea is classified basically into two groups: the chingcha group (more commonly called Oolong tea group) and the white tea group (3). The white tea group is simpler and can be further subgrouped into silver tip pekoe, Baimudan, Kungmei and Shoumei. The chingcha group (or Oolong tea group) has a large number of varieties available and this makes the subgrouping more complicated; the bases of classification are also different. Currently, the better-accepted subgrouping is as follows:

Minbei (Northern Fujian) chingcha (such as Wuyi Yancha)
Minnan (Southern Fujian) chingcha (such as Anxi Tieguanyin tea)
Guangdong chingcha (such as Fenghuang Shuixian tea)
Taiwan chingcha (such as Wenshen Pouchung, Dungding Oolong, and Pomfeng tea)

III. MANUFACTURE OF PARTIALLY FERMENTED TEA

Partially fermented tea is considered a special tea combining the processing characteristics of the two big groups of tea, green tea and black tea. Thus, in the manufacture of partially fermented tea, the first steps are based on the processing steps for black tea to develop the characteristic aroma due to fermentation, followed by steps used in the manufacture of green tea to stabilize the color, aroma, and taste. The biggest difference from the other groups of tea manufacturing is in the steps of withering and shaking. These two steps are the most critical steps in the manufacture of partially fermented tea. The shaking step (sometimes called the fine manipulation of tea leaf) is a characteristic step in the manufacture of chingcha. Among the six big groups of tea, fine manipulation of green tea leaf is practiced only in the manufacture of chingcha. This step is also the most precise, tedious, time-consuming, and labor intensive. In comparison, the manufacture of white tea is the simplest among all the tea manufacturing procedures (4).

A. Manufacture of White Tea

Manufacture of white tea is very simple (5). The basic processing scheme is as follows:

Fresh tealeaf → Withering → Drying(air drying, solar drying,

or mechanical drying) → White tea.

White tea belongs to the group of tea that does not require panning, rolling, and shaking. Therefore, its manufacture saves time and labor. However, the selection of raw material in white tea manufacture is extremely stringent; only the plucking of young tea leaf with much fine hair can produce good-quality white tea with lots of pekoe.

1. Manufacture of Silver Tip Pekoe Tea

a. Plucking of Fresh Tea Leaves Silver Tip Pekoe is the highest quality grade among white tea and can be made only from the young tender tea leaf. The tea leaf usually is plucked from the single, well-developed leaf shoots of the Dabaicha or Shuixian tea cultivars. Or, after the young leaves with "one bud (needle/shoot) and two leaves" are plucked, the shoot (needle) is pulled out and used as the raw material for making Silver Tip Pekoe. In plucking the tea leaf for making Silver Tip Pekoe, a sunny day is important. The best time is when the air is cool and dry. The plucking technique is very stringent, with ten not-to-pluck guidelines—for example, not to pluck tea leaf shoots after rain, shoots covered with dews, skinny shoots, violet shoots, damaged shoots, banjhi leaves, distorted shoots, and others. The shoots, after plucking, cannot be pressed. The plucking season is only before and after Ching Ming festival (early April).

b. Processing Technology Undisturbed withering for long period of time is the most critical step for manufacturing Silver Tip Pekoe. On a sunny day, the tea shoots can be spread lightly on withering trays, with even spreading and no piling, and left in the sun to wither and dry. The tea shoots must not be turned over, to avoid reddening of the tea shoots. In general, withering and drying in the sun for one day will achieve 80–90% dryness. The shoots are then dehydrated with low heat to finish the drying process. During the drying process, a sheet of white paper is put on top of the pile to avoid shoots burning and turning yellow. The drying temperature is 40–45°C. The load is about 250 g for about 30 mins. Another procedure is to keep the tea shoots in a ventilated environment to wither to 70–80% dryness. They are then moved to finish drying under the hot sun. This process will take 2–3 days to complete. When manufacturing Silver Tip Pekoe on rain days or low-temperature and high-relative-humidity weather, sun-drying for one day can achieve only 60–70% dryness. The shoots have to be sun-dried again the next day to 80–90% dryness, followed by low heat dehydration to finish the drying process. In case 60–70% dryness cannot be achieved, or when the next day is rainy, it is then necessary to dehydrate at 40–45°C with low heat in the same evening or early next morning to complete dryness. After tea shoots are harvested, in case the weather is rainy and the shoots cannot be withered and dried under the sun, the shoots can be dehydrated directly with low heat to dryness. However, the quality will be severely damaged.

2. Manufacture of Baimudan, Kungmei, and Shoumei Tea

a. Criteria for the Fresh Tea Leaves Baimudan requires that the fresh tea leaves come from the Dabaicha cultivar. The tender leaves should be "one bud with two leaves" with the "three white" criteria—the shoot is white, and the backs of the first and the second leaf have a thick layer of fine white hair. Length of the shoot should be similar to that of the

leaves, but not shorter. The first plucking of the tender leaves in the spring is considered to be of better quality. Fresh leaves for Kungmei need to have "one bud with three leaves," and Shoumei is made from leaves only, without the shoot.

b. Processing Technology Manufacturing of Baimudan and Kungmei includes the following processing technologies:

Natural Withering Method. After harvesting, the fresh tealeaf is spread evenly on the withering trays, one layer thick without any piling, according to the guideline. They are left on the shelves of the withering room with good natural ventilation for 35–45 hrs, withering without any disturbance. When the fine leaf hair starts to turn white and the leaf color darkens, two trays of withered leaf can be combined into one tray. The withering process continues until the moisture content is 22%, then two trays of withered leaf are again combined into one tray. The withering process continues for another 10 hr, until the moisture content is about 13%. This is the end of the withering process, resulting in the best quality Baimudan. The withering process is affected by the temperature and relative humidity in the environment and has to be adjusted accordingly based on the degree of withering. According to practical experience, the total withering time in the room is about 48–72 hr. When the weather changes during the withering process with the occurrence of humidity and low temperatures, tea leaves with 80% withering can be taken out from the trays, spread and piled up accordingly, with the more withered leaves piled thicker and the less withered leaves piled thinner. If the withering is only 60–70%, they should be dehydrated twice, with the first dehydration at 100°C to about 80–90% withering, followed by spreading and cooling and again dehydrated at 80°C to complete the process. When the withering is completed within 24 hr, the degree of withering is incomplete; or, when the dehydration starts with leaves losing only 40% of their weight, the color of the final product is yellowish green with a green astringent odor. These products do not meet the quality criteria of white tea. When the withering period is extended over 72 hr because of a low degree of withering, the product shows dull dark color with poor aroma and, in some cases, even a moldy odor.

Heated Withering. Heated withering consists of applying hot air to the withering room to conduct the withering process. The temperature should be controlled within 22–27°C, with relative humidity of 60–75%. The process should be carried out for 25–30 hr, with moisture content of the tea leaf reduced to about 25%. At this stage, the leaves are bright green with the tip sticking up and the leaf circumference curling, imparting a pricking sensation to handfeel; they should be unloaded from the trays and piled up for 3–4 hr. The main leaf vein turns reddish brown and leaf color turns dull green, accompanied by disappearance of greenish odor and the appearance of a fresh sweet aroma. Another dehydration for 25 min at 80°C in a low-temperature dryer will complete the process. Higher temperature will turn the hair color from white to yellow. This kind of heated withering method in a room can produce Baimudan with the traditional quality characteristics equivalent to those produced by the natural withering process. But the time to wither can be considerably decreased and not affected by the weather.

3. Manufacturing Method for New White Tea

The manufacturing method for new white tea is basically the same as the manufacturing method for Kungmei within the traditional white tea category. The main difference is on the piling, slight rolling, and rying steps following suitable withering.

a. Criteria for Fresh Tea Leaf New white tea is considered a medium to low-grade product. The degree of tenderness for the fresh tea leaf is lower than that required for Kungmei white tea.

b. Processing Technology The general processing scheme for new white tea is as follows:

Fresh tea leaf → Withering → Piling → Rolling → Drying

Withering. Withering for new white tea can be achieved by natural withering, heated withering in a withering room, or withering in a withering trough. Under normal weather condition, natural withering is generally conducted. The fresh leaves are withered on a withering trough or tank for 24–36 hr until the moisture content in the withered leaves is 25–30%.

When heated withering in a room is conducted, the temperature in the room is maintained at 8–10°C higher than outside. Withering time is 18–24 hr. This process is suitable for rainy weather.

When heated withering in a withering trough is conducted, temperature is maintained at 24–36°C. The fresh leaves are piled to 8 cm deep with turnover every 30–60 min for 12 hr. Withering quality is lower for this process and is seldom used.

Piling. When withering reaches a suitable stae, the tea leaf is taken out from the trays and piled on the floor to 30 cm deep but not pressed. This depth may vary depending on the temperature and relative humidity in the environment. The piling area should have good ventilation with temperature of the pile controlled at 2–4°C higher than room temperature. The piling time is 2–6 hr until the leaf veins turned reddish brown, and leaf color turns from light grayish green to dark grayish green or brown, with disappearance of greenish offensive odor and appearance of sweet aroma.

Rolling. At a suitable stage of piling, the tea leaves are put inside the rolling machine to roll for 10 min without pressure, until tea leaves show slight curling with part of the leaf juice being squeezed out.

Drying. Tea leaves are dried in a dryer at 100–120°C to a moisture content of 5%.

B. Manufacture of Chingcha (Oolong-type Tea)

Chingcha is also called Oolong tea. Oolong tea has enjoyed a worldwide reputation for a long time, and it is also called the "pearl" among the different famous teas. After brewing, Oolong tea has a rich flowery (fragrant) and fruity aroma, induced completely from the different tea cultivars, season, and special processing techniques (6). The taste of Oolong tea is rich, with a sweet aftertaste. Drinking Oolong tea has a special sensation—a tonic like effect: after the tea liquor passes the throat, it has an aftertaste, and when this taste is evaluated carefully, it is like having something to chew on. Due to the different production areas and cultivars, various Oolong teas have their own sensory characteristics. Wuyi Yancha tastes sweet, pure, and fresh, with a sweet aftertaste. A concentrated drink will not give a bitter taste. This special tone is called Wuyi Yan tone, or sometimes soymilk tone. The taste of Anxi Tieguanyin is rich and smooth. A concentrated drink gives a slightly bitter and astringent taste with a long-lasting sweet aftertaste. This characteristic is called Guanyin or Yin tone. The circumference of the back of Oolong tea leaf shows a reddish-brown color with light green color in the center, forming a characteristic "green leaf with a red circumference." This is typical of Oolong tea processing technology and is typical of partially fermented tea. The manufacturing process for Oolong tea is different from that for black or green tea and is much more complex. It technology utilizes the processing principles for fermented black tea and the unfermented green tea. The process does not completely destroy the leaf structure, but slightly injures the circumferences of the leaves through their rubbing each other. It requires that the cell components not change completely but induce a

partial oxidation; and through this complicated technological process, the characteristic color, aroma, and taste of Oolong tea are produced (6).

A typical processing scheme for Oolong tea is as follows:

Fresh tea leaf → Solar withering (as shown in Fig. 2) → Indoor sitting and shaking (as shown in Fig. 3) → Panning (as shown in Fig. 4) → Rolling (as shown in Fig. 5) → Drying (as shown in Fig. 6)

However, because of the slight variations in processing procedures, different Oolong teas have their own characteristic flavors. Based on their typical quality characteristics, Oolong teas can be classified into four big groups with six typical examples, as follows:

Minbei (Northern Fujian) chingcha: Wuyi Yancha
Minnan (Souther Fujian) chingcha: Anxi Tieguanyin
Guangdong chingcha: Fenghuang Shuixian
Taiwan chingcha: Wenshen Pouchung, Dungding Oolong, and Pomfeng tea

The degrees of fermentation of various Oolong teas are as follows:

Pomfeng tea > Wuyi Yancha > Anxi Tieguanyin > Dungding Oolong > Fenghuang Shuixian > Wenshen Pouchung.

1. Manufacture of Wuyi Yancha

The manufacturing method for Wuyi Yancha is very special and uses fine technology. It includes the essentials for making black and green teas. The manufacture includes proper selection of tea cultivar with optimum plucking, and at the same time, the application of very detailed heating (baking) technology. The degree of fermentation for Wuyi Yancha is more extensive than for Anxi Tieguanyin and Fenghuang Shuixian. It is a tea made with extensive withering and shaking. Except for the highest quality grade that still employs the

Figure 2 Solar-withering is the first step in the manufacturing of Oolong-type tea.

Figure 3 Indoor-sitting and shaking is the second step in the manufacturing of Oolong-type tea.

traditional manual processes, Wuyi Yancha is now mechanically processed using equipment such as withering trough, shaking machine, panning machine, rollers, and dryers. The manufacturing processes are simplified into withering, shaking, blanching, rolling, preliminary (primary) drying, and final drying (7).

a. Solar Withering Fresh tea leaf is evenly spread on withering trays with a loading density of 0.6–1 kg per square meter. These trays are loaded onto the withering shelves for solar withering. In order to have even withering, when the tea leaves are withered to a certain degree, they are gently piled and turned over manually without damaging the leaf. When the

Figure 4 Panning is the third step in the manufacturing of Oolong-type tea.

Figure 5 Rolling is the fourth step in the manufacturing of Oolong-type tea.

sunlight is strong during noontime, it is not appropriate to wither the tea leaf under the sun. With tea leaf harvested on rainy days or in the evenings, traditionally it can be withered with heating indoor. With mechanical processing, tea leaf can be heat-withered in the withering trough. The time for solar withering depends on conditions of the tea leaf and weather condition. The time is shorter with stronger sunlight and longer with weaker sunlight. In general, it is within 2 hours. When a heated withering trough is employed, temperature is controlled between 32 and 38°C for 40–70 min with depth of tea leaf between 15 and 18 cm.

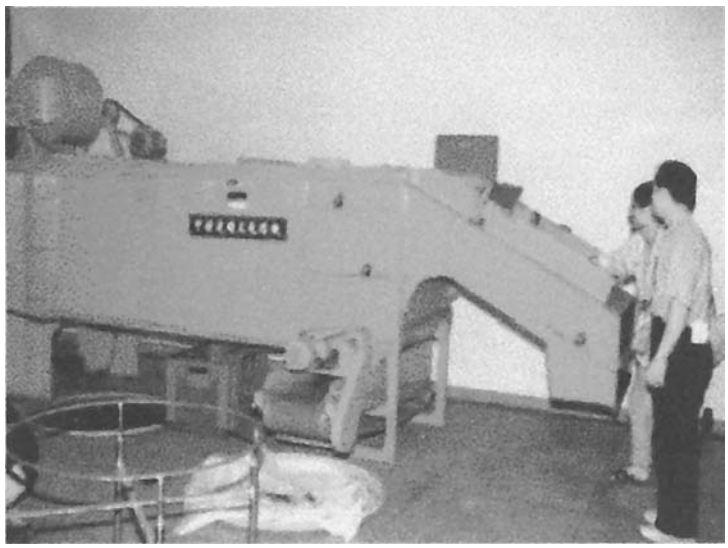

Figure 6 Drying is the last step in the manufacturing of Oolong-type tea.

Termination of solar withering is determined by a weight loss of 10–15% of the original weight of fresh tea leaf (i.e., the tea leaf retains about 65–70% of its moisture).

b. Indoor Sitting and Shaking This is a quality-determining step in the manufacture of Wuxi Yancha. Sitting and shaking are conducted alternately. During shaking, the tea leaf moisture evaporates gradually and softens the tea leaf, whereas during sitting, moisture in the leaf veins diffuses to the rest of the leaf and hardens the tea leaf. During sitting and shaking, the windows in the room have to be closed, maintaining a stable temperature between 22 and 27°C and a relative humidity of 80–85%. When room temperature is lower than 20°C, such as in spring with cool temperature and rainy days, supplemental heating is needed. Shaking causes the leaves to roll and turn over on the trays, allows the leaves themselves and leaf and tray surface to rub against each other, damages the cells in the leaf circumference, and induces partial oxidation and reddening. In general, the first shaking is conducted after sitting for 1 hour, followed by hourly shaking for 7–9 times.

In mechanical shaking, the shaker is 160–200 cm in length with a diameter of about 60 cm. It has a central axis. The body of the shaker is made of bamboo or wire mesh with a wooden or iron frame. Rotation is 10–25 times per minute. Each load weighs 10–12 kg. Shaking lasts 2–6 min for every 30–60 min, with a total of 8–12 shakings. Mechanical shaking is more efficient than manual operation and is more suitable for mass production.

Tea leaf that has undergone proper sitting and shaking has the following indicators: spoon-shaped leaf, with light green surface and bright reddish color circumference, leaf apex is prickly, "sa-sa" sound when turned over lightly, transparent veins, disappearance of greenish odor, light fragrant aroma, moisture content about 65%.

c. Panning and Rolling Traditional manufacturing process includes two panning and two rolling steps. Panning is conducted in a slanted pan with diameter of 60 cm and 25° slanting. Primary panning temperature is 180–220°C. Each load weighs 0.5–1.0 kg. After the tea leaf is loaded, it is turned rapidly with both hands. Panning lasts for 5–7 mins until the tealeaf is soft and sticky to the hands, with disappearance of greenish odor and appearance of pleasant aroma. After the primary panning, tea leaf is transferred to the roller for rolling when tea leaf is still hot. This primary rolling time is 2–3 mins. Leaf juice is leached out with the tea leaf rolled to a stringy shape. This tea leaf mass is broken up and again panned at 150–180°C. This secondary panning is basically a stewed panning until the tea leaf is too hot for the hands. The tea leaf is again rolled for a short time with pressure in order to tighten the stringy shape.

The mechanical process uses a blanching machine and rolling machine to conduct the panning and rolling processes, and the procedure is simplified to one panning and one rolling. Blanching can be conducted in either rolling-drum-type or pan-type blancher. The process should be conducted at high temperature, rapid and more stewed than ventilated panning. Rolling is generally conducted in a small rolling machine requiring high pressure, and rapid, hot rolling for 5–6 min. The tea leaf mass can then be disintegrated for drying.

d. Drying Wuyi Yancha is generally mechanically dried in a two-step process. The primary drying is at 120–130°C, with a loading density of 2 cm thick, and a final 70% dryness. The tea leaf is then spread to cool for 1–2 hr. The final drying is at 80–90°C with a loading density of 3 cm thick until fully dried.

2. Anxi Tieguanyin

Both Anxi Tieguanyin and Wuyi Yancha belong to the Chingcha (Oolong tea) category. The origin is Anxi Xian of Fujien Province. This tea was first developed during 1723–1735

and is one of the traditional Chinese teas. The appearance is like a curly string, heavy like iron, beautiful as the Goddess Guanyin, and thus called Tieguanyin (meaning an ironlike Guanyin). It has long been considered a premium chingcha. Its quality characteristic is strong and orchid-type long-lasting aroma. The taste is sweet, smooth, and fresh with a special tone, called Yin tone. It is well appreciated by consumers (8).

a. Plucking of Fresh Leaves Tieguanyin cultivar is the best for its manufacture. When the apical bud forms the latent bud, the plucking should be the new "one bud with 2–3 leaves," with "one bud with 3 leaves" being the best. After harvest, the leaf should be separated, spread, and panned accordingly.

b. Manufacture Scheme for Tieguanyin Tea Tieguanyin is produced according to the following manufacture scheme:

Fresh tea leaf → Solar withering → Indoor sitting and shaking
(conduction of partial fermentation) → Panning → Rolling
→ Primary drying → Rolling in a bag → Baking in a bag
→ Disintegrating the mass → Secondary drying → Refining
→ Final drying → Tieguanyin

Solar Withering. Solar withering is usually conducted at 3 P.M. in the afternoon on the same day of harvesting of tea leaves. This is because the solar rays start to weaken at this time and this is beneficial for homogeneous solar withering. The procedure consists of spreading the leaf lightly with leaf thickness (loading density) of 0.6–1.0 kg per square meter. The leaves are withered to the stage with the disappearance of shininess on leaf surface, a color change from bright green to dull green, dropped-down leaf apex, and softening of leaf texture. With overcast or rainy weather, the leaves can be withered indoors naturally or with supplementary heating.

Indoor Sitting and Shaking. Withered tea leaf is left sitting for 1 hour before shaking. It takes four or five shakings, with pressure lighter at the beginning and heavier toward the end. The sitting time is shorter at the beginning and longer at the end. The spreading thickness is thinner at the beginning, followed by thicker spreading later. The total time is about 8–12 hr.

The first shaking starts after solar withering for 40–60 mins and continues for 2–3 min. The withered leaf is left sitting for 1–1.5 hr with leaf-spreading thickness of 7–10 cm. The second shaking is 5–7 min, followed by a sitting of 1–1.5 hr with leaf-spreading thickness of 10–13 cm. The third shaking is the critical step with the appearance of strong green leaf odor as appropriate. The shaking time is 8–12 min. The tea leaf is then left to sit for 3–5 hr with leaf-spreading thickness of 13–15 cm. After three shaking steps, if the circumference tea leaf turns reddish, the leaf surface is yellowish green with red spots, and the greenish odor is replaced by a flowery (fragrant) aroma, it is considered to have achieved the proper shaking. If not, the tea leaf has to be shaken for the fourth or fifth time.

The shaking process can also be achieved using drum-type shakers with rotation speed of 5–20 rpm. The tea leaf has to be shaken 4–5 times with shaking and sitting times the same as the manual process.

Panning. Panning is now achieved with a drum-type blancher at 260°C for 7–10 min. (The manual panning process can also be applied, but it is more complicated and is now seldomly used.) The indicators for proper panning are slight dehydration of leaf circumference with pricking handfeel sensation, color change from bright green to dull green, and moisture content at 64–67%.

Rolling and Baking. Rolling and baking are conducted alternatively with various preliminary rolling, preliminary baking, first rolling-in-cloth-bag, second baking, and second rolling-in-cloth-bag steps. Preliminary rolling is to pressure-roll the hot tea leaf coming out from the blancher for 3–5 min. When the tea leaves form a string shape, they are ready to be broken apart for the first baking. The first baking process is to bake the tea leaf after the first rolling at 100–120 C to 60% dryness with the string-shaped tea leaf not sticky to handfeel. The tea leaf is then ready for the following "rolling-and-baking-in-cloth-bag" process. The bags used are made with 70 cm × 70 cm white cloth. One kilogram of the hot tea leaf from the first baking is wrapped per piece of cloth. The bag is tightly held with one hand, followed by rolling on a platform with the other hand. This process is commonly called "rolling-and baking-in-cloth-bag." In this rolling process, pressure is applied lightly at the beginning followed by heavier pressure as it proceeds. The rolling time is about 10–20 min, until the tea leaf turns into curly snail-shape tight strings. The tea leaf mass is then taken out from the bag and let cool.

The procedure to manufacture Tieguanyin is a special one. The withered tea leaf is first baked to partial dryness, wrapped in a cloth bag, hand-rolled lightly to a big tea leaf ball in the bag and followed by low-temperature (less than 100°C) slow baking to tightly curl up the tea leaf. This process, commonly called "rolling-and-baking-in-cloth-bag," is repeated a few times. It utilizes the baking heat to change the components in withered tea leaf into aromatic and tasting compounds. These compounds are long lasting even after a few brewings with a repeated aromatic and smooth tone. Due to shortage of labor in recent years, the "rolling-and-baking-in-cloth-bag" process is commonly conducted using a cloth-ball roller to simplify the special repeated rolling and baking processes. The mechanical process can produce Tieguanyin that has a good aroma but lacks the proper taste and throat-soothing tone. Its deficiency is the lack of traditional Yin tone in Tieguanyin.

3. Manufacture of Fenghuang Shuixian Tea

a. Plucking of Fresh Leaves There are many good cultivars in the Fenghuang (Pheonix) mountain in Guangdong Province. The tea farmers there selected single clones suitable for manufacturing of good quality tea. They grow this single clone and pluck only this same clone for Fenghuang Shuixian tea manufacture. Shuixian is a more popular cultivar. In general, plucking is conducted four times a year, (spring summer, fall and winter). Tea made from spring plucking is more common and has the best quality. Plucking criterion is to pluck the new shoots 3–5 days after latent buds are formed properly (8).

b. Manufacturing Technology For the manufacture of Shuixian tea, the plucking is usually conducted in the afternoon, followed by solar withering in the early evening (late afternoon) and other processing steps throughout the night. The manufacture procedures include solar withering, indoor sitting and shaking, panning, rolling, and drying.

Solar Withering. Tea leaf is spread thinly, 0.6–1.0 kg per square meter. Leaves should not be piled up each other. During the withering process, there is no need for shaking or turnover. Leaf is solar withered until the leaf loses its luster or brightness, leaf color changes to dull green, leaf texture softens with appearance of light, pleasant aroma, and moisture content is lowered to 70%. This usually will take 10–30 min.

Indoor Sitting and Shaking. This is a more important step and takes 8–12 hr. The procedure is to pile up the fresh leaf with the center being lower than its surroundings. These piles are loaded onto withering shelves in the withering room, with temperature controlled at 22–26°C and relative humidity at 70%. The sitting and shaking procedures are repeated

4–5 times, with 5–10 min of shaking each time. The leaf is again piled up as before and left to sit for about 2 hr. Manual shaking should be lighter at the beginning and proceed to heavier shaking. Piling should be thinner at the beginning and progress to thicker piling. Tea leaf is considered properly indoor sit and withered when the following phenomena occur: tea leaf turn 30% red (i.e., 30% red on the circumference with 70% green in the center), leaf veins turn transparent (in front of a light source), leaf surface turns yellow, leaf circumference turns bright red, leaf shape turns spoon-like, showing of "sa-sa" sound when turned over, decrease of greenish odor, increase of fruity (fragrant) aroma, and moisture content reduced to 65–70%.

Panning. Panning should be conducted rapidly at high temperature with more stewed panning than ventilated panning (i.e., retaining more steam in the pan, without ventilation if possible, for better heat transfer). Panning should be done swiftly, with even turnover, and avoided burning on the tea leaf.

Rolling. Rolling is conducted in two steps. The first rolling is right after panning and the second rolling is conducted after the second panning. Tea leaf is generally hand-rolled. With a larger quantity, tea leaf can be rolled in a small-scale tea roller. Rolling is conducted lightly until the leaf is tightened into strings.

Drying. Drying procedure for Fenghuang Shuixian is the same as that for Wuyi Yancha.

4. Manufacture of Pouchung and Dungding Oolong Tea

Wenshen Pouchung tea and Dungding Oolong tea are typical examples of Pouchung-type tea. They have lighter degree of fermentation among all the partially fermented teas. These teas have only 8–18% of their polyphenols oxidized and have a dark green appearance. Color of brewed liquor ranges from honey green to honey yellow, or golden yellow. These teas are bright and transparent, and their taste is lively, sweet, and smooth. Aroma is fresh pure, and flowery (fragrant). Wenshen Pouchung tea is mainly produced in northern Taiwan. It has a string-shaped appearance. The degree of fermentation is lightest among all the partially fermented teas. Dungding Oolong tea is semispherical, and its fermentation is more extensive than Wenshen Pouchung tea. It is a tea produced with very fine and complicated techniques. The annual production of Wenshen Pouchung tea is about 1500 metric tons, and that of Dungding Oolong is 15,000 metric tons. They are the mainstream teas in the consumer market in Taiwan (9).

a. Manufacture Schemes

Wenshen Pouchung Tea

Fresh tea leaf → Solar withering → Indoor sitting and shaking
(conduction of partial fermentation) → Panning → Rolling
→ Disintegration of tea mass → Primary drying → Redrying
→ Wenshen Pouchung tea.

Dungding Oolong Tea or Semispherical Pouchung Tea.

Fresh tealeaf → Solar withering → Indoor sitting and shaking
(conduction of partial fermentation) → Panning → Rolling
→ Disintegration of tea mass → Primary drying
→ Mass rolling 3–5 times with repeated panning → Redrying
→ Dungding Oolong tea or Semispherical Pouchung tea.

b. Seasons for Manufacture In Taiwan, Pouchung teas are best produced in the spring and winter seasons, followed by autumn. Pouchung tea produced in the summer is too bitter and astringent.

c. Suitable Cultivars In Taiwan, the better cultivars for Pouchung tea are Chin-shin Oolong, TTES No. 12, TTES No. 13 and Syh-Jih-Chun, followed by Chin-shin Da-pang cultivar.

d. Manufacture Methods

Selection of Raw Materials. The raw materials for Pouchung teas should be the young tender leaves with soft texture, thick, and light green in color. The leaf should be banjhi leaves. Younger leaves produce brew liquor with stronger bitter and astringent taste and dull green color, whereas older leaf produces brew liquor with light taste and its leaf is yellowish green.

With overapplication of nitrogen fertilizer, the fresh leaf is dark green and has high moisture content. The final product has a darker color with lighter aroma.

Fresh leaf from different sources (such as different cultivars, morning-plucked leaf, afternoon-plucked leaf, or evening-plucked leaf) should be separated and processed separately in order to control quality.

When temperature in the environment is high, suitable quantity control of intake fresh leaf is critical in order to avoid reddening of fresh leaf due to high temperature and muggy environment, resulting in "dead" leaf.

Solar Withering. The purpose of solar withering is to reduce the moisture content in cells of tea leaf by evapotranspiration of the water in the cells as accelerated by the solar heat. At the same time of reduced water activity, the cell membrane loses its semipermeability capability. The different components in the cells (particularly the catechins) are oxidized by its enzymes and proceed with its fermentation.

The procedure for solar withering is to spread out the fresh leaf on cloth trays or withering trays to conduct withering under the sun, with loading density of 0.6–1.0 kg per square meter. Temperature on the tea leaf should be maintained at 30–40°C. When the solar temperature is above 40°C, the tea leaf should be protected with shading networks to avoid sunburn of the leaf, causing "dead" leaf. During the withering process, depending on the rate of moisture evapotranspired from the fresh leaf, the leaf should be turned over 2–3 times to provide even withering. Withering time usually is 10–20 min. It can be extended to 30–40 min when the sunlight is weak. This is dependent on the rate of water evapotranspiration.

The degree of solar withering is observed visually. When the luster of second leaf (or first leaf of banjhi leaves) disappears, the leaf will show a wavy appearance, giving a soft handfeel. The greenish odor disappears, with appearance of pleasant (fragrant) tea aroma. This is considered proper solar withering. The fresh leaf loses 8–12% of its weight.

Indoor Sitting and Shaking. The purpose of indoor sitting and shaking is to continue the fermentation action induced by solar or hot air withering. The tea leaf continues to ferment and induce the complicated chemical reactions that form the characteristic taste and aroma of Pouchung tea. Shaking is conducted by lightly turning over the tea leaf with both hands so that the circumferences of the tea leaf can rub against each other and in consequence the cells around the circumference will be damaged. By doing so, air can enter the cells easily and induce fermentation; at the same time, water can be evapotranspired evenly due to this turnover.

After solar or ot air withering, tea leaf is moved into the indoor withering room and spread on trays with a loading density of 0.6–1.0 kg per square meter. The tea leaf is left sitting for 1–2 hr. Due to loss of moisture, leaf circumference shows a slight wavy shape. The

first shaking is then initiated with light motion for about 1–5 min. The shaking activity is more vigorous with further shakings and increase in shaking time. Leaf thickness for subsequent spreading increases with longer sitting time. In general, tea leaf will be shaken 3–5 times, followed by 90–180 min sitting after each shaking. The last shaking usually is around midnight with sudden drop in temperature, thus the spreading should be thicker. During early spring or winter, the temperature is usually lower. Tea leaf, after shaking, is usually piled up to 60 cm or higher in bamboo cages for the sitting period. This procedure will increase the leaf temperature and thus the fermentation reaction with production of characteristic taste and aroma. The sitting after the last shaking is 120–240 min with disappearance of the greenish odor and production of pleasant tea aroma. The leaf is then ready for panning.

The first and second shaking should be light, just turning over the tea leaf. If the shaking is too severe at the beginning, the leaf can be easily damaged, causing dull, dark appearance with bitter taste for the brew liquor. If the shaking is inadequate, the aroma of Pouchung tea is not strong enough, with off-greenish odor in extreme cases. Therefore, indoor withering time and number of shakings should be appropriately adjusted depending on the tea cultivar, nature of the green tealeaf, season, and weather condition.

Panning. The purpose of panning is to inactivate the enzymes by high temperature, inhibiting further fermentation and guaranteeing the characteristic taste and aroma of Pouchung tea. Tea leaf loses large amount of moisture during panning and thus softens, making the rolling into string shapes and dehydration easier.

Panning can be conducted using a rotary pan or panning machine. Temperature of the rotary pan surface should be 160–180°C, or 250–300°C on the temperature indicator of the panning machine. At the beginning of panning, there is a "paat-paat" sound. The panning period depends on the nature of tea leaf and loading quantity. At the end of the panning process, tea leaf has no greenish odor, leaf texture is soft and pliable to hand-holding with a strong pleasant (fragrant) aroma. It is critical not to overpan Pouchung tea: this results in pricky leaf circumference or burnt odor. Tea leaf unloaded too early from the pan will be underpanned and have a greenish odor and red central vein.

Rolling.

Rolling of Wenshen Pouchung tea. After being unloaded from the pan or panning machine, the tea leaf is turned over by hand 2–3 times to release the hot vapor in tea mass, followed by loading into the drum of the rolling machine (roller) to proceed with the rolling process. Pouchung tea does not have critical appearance requirements for its leaf apex or white hair; therefore, the leaves can be rolled slighter heavier. Larger leaves can be rolled twice to improve the appearance. The first rolling is 6–7 min, followed by breaking up the tea mass to release the hot vapor. The second rolling of 3–4 min is heavier and improves the appearance.

Rolling of Dungding Oolong tea. Dungding Oolong tea has to go through a special mass rolling process in order to produce its characteristic flavor and appearance. During mass rolling, control of repanning temperature, rerolling pressure, and moisture content of tea leaf has significant effects on the appearance and flavor of this semispherical-type Pouchung tea. The rolling process is semispherical-type Pouchung tea manufacture includes three distinct steps: preliminary rolling, primary drying, and mass rolling (repeated rolling in bag and panning).

Preliminary rolling. The preliminary rolling for Dungding Oolong tea is the same as that for Wenshen Pouchung tea.

Primary drying and sitting for rehumidifying. After this preliminary rolling, the tea leaf goes through a primary drying by tea dryer. When held in the hands, the tea leaf is soft and pliable but not sticky (commonly called semidried with about 40–50% moisture content). The leaves are again left to sit on trays until the next day for mass rolling.

Mass rolling. After primary drying and sitting for rehumidifying tea leaf is loaded to rotary panning machines for heat-softening. When the center of the leaf is heated to 60–65°C, the leaves are loaded into special cloth bags for manual or mechanical mass rolling. Mass rolling should be repeated by opening the bag at a suitable time, disintegrating the tea mass, repanning, and mass rolling the tealeaf again. Moisture evaporates gradually from the tea leaf which becomes more compact.

Drying. The purpose of drying is to inactivate the residual enzyme activities, terminate further fermentation, and at the same time stabilize tea leaf quality. Drying is conducted by a tea dryer. This procedure will improve the aroma and taste of tea leaf, remove the greenish off-odor, reduce the astringency, and give the brew liquor a clear and beautiful color. The moisture content in tea leaf is reduced to 4%. Both volume and weight are reduced. This is convenient for packaging, storage, and transportation.

Chain-belt–type tea dryers are generally used for this drying process. Incoming hot air at 100–105°C is introduced to dry the tea leaf for 25–30 min. This is dependent on the capacity of the dryers (tea leaf thickness of 2–3 cm). Overmatured leaf can be dried twice to improve the appearance. The procedure is to dry the leaf for 10–30 min first, then spread to cool, and at the same time soften the tealeaf. The tea leaf is dried again to improve the stringy appearance at 90–95°C.

5. Manufacture of Pomfeng Tea (Taiwanese Oolong Tea)

Pomfeng tea is a typical Taiwanese tea. The generally so-called Taiwanese Oolong tea is a Pomfeng tea. It has the highest degree of fermentation among all the partially fermented teas. 50–60% of the catechins oxidized. It has a beautiful appearance, with white, red, yellow, brown, and green colors alternating like a flower. The higher quality Pomfeng tea even has white hair. The brew liquor has an amber color, with a smooth, sweet, and soothing taste carrying a ripe fruity aroma. Taiwanese Pomfeng tea from Hsinchu County and Miaoli County is the most well known. Annual production is less than 50 metric tons. Its availability is limited and it is an extra-precious tea.

a. Manufacture Scheme The manufacture scheme for Pomfeng tea is as follows:

Fresh tea leaf → Solar or hot-air withering → Indoor sitting and shaking
→ Panning → Wetting and softening → Rolling
→ Disintegrating tea mass → Drying → Product.

b. Manufacture Seasons Pomfeng tea is best made between the 10th day of the sixth month to the 20th of the seventh month (lunar calendar) of each year.

c. Cultivars Among all the tea cultivars in Taiwan, Ching-shin Dapang is the most suitable cultivar for this type of tea.

d. Manufacture Procedures Manufacture of Pomfeng tea is similar to that for Wenshen Pouchung tea. The biggest difference is that Pomfeng tea requires more extensive withering and shaking. It also a "wetting" or "rehumidifying" treatment after panning that is an extremely rare procedure in tea manufacture.

Raw Fresh Tea Leaf. Raw fresh tea leaf for Pomfeng tea manufacture is selected from the "one bud with two leaves." The shoot should have a bigger bud with more white hair and soft texture. The higher quality Pomfeng tea is even selected from "one bud with one leaf" after infection with tea green fly (*Empousca flavescens* Fabricus). After receipt of the raw materials at the factory, procedures for the handling of green tea leaf and other critical points are the same as that for Pouchung tea.

Solar Withering. Solar withering for Pomfeng tea is longer than for other partially fermented teas, with more extensive withering. The proper degree of withering is determined by the disappearance of luster on the leaf surface, a wavy appearance, surface wrinkling of the tender veins due to loss of moisture, and dropping of the bud and first leaf. Tea leaf loses 25–35% of its moisture at the end of withering.

Indoor Sitting and Shaking. The number of shakings and pressure applied are more extensive as compared to other Pouchung tea. The first and second shakings are light, avoiding damage to the leaf with improper loss of moisture. The better phenomenon is called "holding the moisture"—the tea leaf does not ferment properly, with development of blackish brown color on the leaf surface and a poor appearance as well as lack of bright brew liquor color. When indoor withering is conducted properly, with proper loss of moisture, the circumference of the tea leaf shows a reddish brown color and the bud is silvery white. When one-third to two-thirds of the leaf surface turns reddish brown, with development of a ripe fruity aroma, the tea leaf is ready for panning.

Panning. Withering for Pomfeng tea is more extensive, thus the tea leaf has less moisture before panning. Panning temperature therefore is lower as compared to other Pouchung tea. The heat applied is only about 80% of that for other Pouchung tea. Panning is appropriate if it resilts in loss of greenish odor, development of ripe fruity aroma, and bud turned silvery white. When tea leaf is held in the hand, it gives a prickly sensation from the slightly dried leaf circumference.

Wetting and Softening. Wetting and softening procedure is typical of Pomfeng Oolong tea manufacturing. After panning, the tea leaf is wrapped in a wet cloth previously soaked in sanitary water and left to sit for 10–20 min. This enhances auto-oxidation and softens the tea leaf, resulting in loss of dryness, brittleness, and pricky sensation to the hand. This procedure helps shape-formation, avoidance of broken leaf, and damage to bud during rolling.

Rolling. Pomfeng tea does not require a tight, stringy appearance. However, it requires even rolling, undamaged bud, and presence of white hair. Therefore, heavy pres-

Table 1 Comparison of Different Manufacturing Procedures in the Production of Partially Fermented Tea

Raw materials	Withering	Degree of fermentation	Panning and rolling	Examples
Fresh tea leaf	Light	8–12%	Single, single	Wenshen Pouchung
Fresh tea leaf	Light	15–30%	Double, double	Wuyi Yancha Dungding Oolong Fenghuang Shuixian Tieguanyin
Fresh tea leaf	Heavy	<10%	No, no	White tea
Fresh tea leaf	Heavy	50–60%	Single, rehydrate, single	Pomfeng tea

sure should not be applied during the rolling process. The rolling process should also be short.

Drying. Hot-air drying of Pomfeng tea is 10°C lower than for other Pouchung tea and at the same time the drying time should not be too long. It is generally accomplished by drying once.

6. Comparison of Different Partially Fermented Teas

Manufacturing procedures differ in regard withering, degree of fermentation, and rolling procedures; the major differences in the manufacture of partially fermented tea are listed in Table 1 (10).

IV. QUALITY EVALUATION AND CHARACTERISTICS OF PARTIALLY FERMENTED TEA

Due to differences in the manufacturing processes, each tea has its own quality characteristics. These differences in quality characteristics subsequently affect consumer preference significantly and also account for the wide variations in unit prices. It is difficult to describe the general characteristics of different teas. However, the differences can be discriminated by sensory evaluation. In other words, different groups of tea have their own characteristics. Green tea is typical example of unfermented tea. The fresh tea leaf is heat blanched at the very beginning to prevent the oxidation of polyphenols. Therefore, the characteristic of green tea is "clear brew liquor with green leaf." From the standpoints of manufacture procedures and quality characteristics, green tea is one that is most ture to its original taste and closest to nature. Yellow tea belongs to another group of unfermented tea. The fresh tea leaf is heat blanched, followed by a step of "piling fermentation to turn leaf yellow." This induces partial auto-oxidation of the polyphenols, with a significant decrease of the catechin esters [epigallocatechin gallate (EGCG) and epicatechin gallate (ECG)], thus causing yellow tea to taste smooth and free of greenish odor, with the characteristic "yellow brew liquor with yellow leaf." Due to the complete withering, rolling, and fermentation processes for fully fermented black tea, the polyphenols are adequately oxidized to form the theaflavins and thearubigins oxidized polymers, thus creating the characteristic "red brew liquor with red leaf" in black tea. In the manufacture of dark green tea, the fresh tea leaf is heat-blanched just like in the making of green tea and is followed by a post-fermentation of pile fermentation, causing nonenzymatic oxidation. Thus, the characteristic of dark green tea is "yellow brew liquor with black leaf."

Chingcha (Oolong-type tea) possesses the characteristics of both the unfermented green tea and fully fermented black tea. In its manufacture are applied the long withering process of black tea manufacture, with shaking to slightly oxidize the polyphenols, followed by the heat-blanching procedures of green tea manufacture to stabilize the oxidized and nonoxidized components in the tea leaf. Therefore, the tea leaf has the characteristic "green leaf with red circumference." Typical brew liquor for chingca is "golden brew liquor with green leaf" with fragrant flowery aroma, and a sweet, smooth, and soothing taste. In addition, various types of chingcha also have their own characteristics. White tea is made through a long withering process. In addition, it is not panned, shaken, and rolled with low-temperature drying. It has, therefore, large amount of free amino acids. In consequence, white tea not only has the characteristic "clear brew liquor with white leaf," its brew liquor also is fresh and smooth. The "showing of aroma with the tea hair" is also another typical characteristic.

A. Quality Evaluation of Partially Fermented Tea

Even with the advances in science and the considerable improvement in analytical methods for tea chemistry in recent years, sensory evaluation of tea quality is still the best for partially fermented tea because of its efficiency, directness and rapidness. Currently, sensory evaluation of tea quality is still preferred worldwide for the following reasons: (a) rapid examination of color, aroma, and taste characteristics, (b) accurate discrimination of tea quality, (c) rapid detection of characteristics n tea quality not determined by other methods, and (d) no requirement for expensive instrument or equipment. However, not all the evaluation methods and special items to be evaluated are the same for the different groups of tea and in different countries. In general, tea quality is sensorily evaluated for its appearance, color of infusion, aroma, taste, and infused leaf. Evaluation methods and items to be evaluated are different for various tea groups. In Japan, the evaluation mainly includes appearance, color of infusion, aroma, and taste, and does not include infused leaf. However, the evaluation of infused leaf and color of infusion for black tea can be very important. In India, black tea is evaluated for its outer appearance, including shape, color, purity, and body, and internal quality, such as tea infusion and infused leaf. The tea infusion is again subdivided into color and taste. Evaluation of infused leaf includes smelling infused leaf aroma or odor and scoring of leaf color. In England, Sri Lanka, and other countries, evaluation includes outer appearance, tea infusion, and infused leaf. Evaluation of outer appearance includes color, evenness, tightness, and amount of white hair bud. Evaluation of tea infusion includes characteristics, color, turbidity, astringency, and aroma. Evaluation of infused leaf includes smelling of infused leaf aroma or odor and scoring of leaf color. As for partially fermented tea, aroma and taste are very important for chingcha. However, outer appearance is very important for white tea—it has the so-called "three white criteria" for its fresh tealeaf: abundance of white hair on its tender bud, first leaf, and second leaf (11).

1. Establishment Requirements for Tea Evaluation

Sensory evaluation of tea quality depends on visual perception, smelling and tasting senses, and handfeel by tea evaluation specialists to determine the tea quality. Accurate determination of tea quality depends not only on the capability for sharp responses on sensory evaluation, matured techniques and experience, but also on the environment and establishment such as the evaluation room, utensils, and water. There are also standardized procedures and criteria to follow for the sampling, brewing, and evaluation methods (12).

a. Requirements for Tea Evaluation Room Lighting for the sensory evaluation room should be natural with homogeneous and adequate lighting, and avoidance of direct sunlight. It is easy to produce reflective lighting under direct sunlight, making it difficult to accurately evaluate the color of tea leaf and its infusion. Changes in light source and its strength significantly affect the evaluation of color. There should not be any reflective or blocking obstacle with red, yellow, blue, green, violet, and other colors besides white and black inside and outside the tea evaluation room. In order to improve the lighting in the evaluation room, the interior of the room should be white in color. Supplemental fluorescent light can also be installed over the evaluation platform. The evaluation room should be dry and clean with fresh air. Ideally, it should be air-conditioned, maintaining room temperature at $23 \pm 1^{\circ}C$ and relative humidity at $50 \pm 5\%$. The evaluation room should be away from the kitchen, toilets, and other facilities that can generate off-odor. There is no smoking inside the room. In addition, the room should not be waxed. In

addition, the room should be kept quiet; talking, laughing, and other noises should be avoided so that the evaluation specialists can concentrate on their task and elevate the evaluation efficiency. Inside the evaluation room, there should be a black-surfaced evaluation platform or table and a cabinet for the evaluation process and storage of tea samples, respectively. Refrigerators should be used to store green tea and partially fermented tea samples.

b. Requirements for Utensils in Tea Evaluation Utensils for tea evaluation must be used for this purpose only. They must be of good quality with uniform standard and perfection, if possible. This will reduce subjective bias to a minimum. Utensils used for tea evaluation are listed as follows:

Tea Tray. Tea trays are used for holding the tea samples for the convenience of sampling for brewing and examination of outer appearance (shape and color). In general, black rectangular or square trays are used (round trays can also be used). Trays should be odorless and tasteless, and can be made of wood, plastic, or metal. Dimensions are 23 cm (length) × 23 cm (width) × 3 cm (depth) and 25 cm (length) × 16 cm (width) × 3 cm (depth) for square trays, for rectangular ones. Each tray can hold 150–200 grams of tea. Tea trays can also be white in color.

Teacup. Teacups are used for brewing the tea and for the evaluation of tea aroma. Cylindrical teacups are made of white porcelain with handle and cover. There is a small hole in the cover. Opposite the handle of the teacup, is a curved or zigzag-shape sprout at the cup circumference. When the teacup is placed on the tea bowl, it is convenient to pour out the brew liquor to the tea bowl. According to the international standard ISO 3103-1980 (E), specifications for standard tea cups are as follows: internal diameter 6.2 cm, outer diameter 6.6 cm, and cup height 6.5 cm with standard deviation of 0.2 cm. The opening opposite to the cup handle is zigzag-shaped. The cover has outer diameter of 7.2 cm, with internal diameter 6.1 cm and a small hole. The volume of each teacup is 150 ± 4 mL.

Tea Bowl. Tea bowls are used to hold the brew liquor for evaluation of its color and taste. They are generally made of white porcelain. ISO 3103-1980 (E) specifies outer diameter of 9.5 cm, internal diameter of 8.6 cm, and bowl height of 5.2 cm. The maximum capacity is 200 mL.

Scale. A scale is used for weighing out the tea samples. In general, a hand scale designed specifically for this purpose is used. It is made of copper. The scale has a rod with a copper cylindrical tray at one end. A copper sheet weighing 3 grams is placed on it for reference. At the other end of the rod is a pointed oblong tray with the shape of small bamboo basket for weighing the tea sample. If this hand scale is not available, a regular weighing balance can be used. Currently, hand-held electronic scales are also used.

Timer. The timer is used to check the tea brewing time. A regular or fixed timer can be used.

Screening Spoon. This spoon is used to screen the small broken tea leaf and is made of fine copper wire or stainless steel.

Evaluation Spoon. This spoon is used to scoop the brew liquor for evaluation. Generally, it is made of silver or nickel-copper alloy with a long handle. The capacity is 5–10 mL. Spoons made of white porcelain can also be used.

Holding Cup. This cup is used to hold the evaluation spoon and screening spoon.

Waste Container. The waste container is used to hold the tasted brew liquor, infused tea leaf, and leftover brew liquor. It can be round or semiround with height of 80 cm, diameter of 35 cm, mid-height diameter of 20 cm. It is generally made of zinc-galvanized iron sheet.

Kettle. A regular aluminum or stainless steel kettle or electric kettle with 5–8 liter capacity can be used to boil the water for tea brewing.

c. Requirement for Water Used in Tea Evaluation Evaluation of tea liquor requires the examination of its color, aroma, and taste after the brewing of tea in water. Thus, water hardness, clarity, and turbidity affect the color, aroma, and taste of the brew liquor. Therefore, poor quality water for brewing affects the accuracy of tea evaluation.

Selection of Water for Brewing. Water from different sources contains different soluble components and therefore affects the quality of tea liquor differently. Water hardness is divided into carbonate hardness and noncarbonate hardness. Water with carbonate hardness produces calcium and magnesium carbonate precipitates during boiling. However, water with noncarbonate hardness does not form these precipitates and is considered good for brewing tea. When brewed with water of carbonate hardness, tea liquor has a black color shade with a light taste, whereas water with noncarbonate hardness does not affect the color and taste of brew liquor that much. Water hardness affects its pH value, and color of tea liquor is very sensitive to pH. When pH value is lowered to 5, it does not affect the color of black tea liquor that much. However, with more acidic water, the color of brew liquor changes from red to reddish yellow. When water is alkaline, with pH above 7, theaflavins are vulnerable to auto-oxidation, causing the color of brew liquor to blacken and lessen its refreshing taste. When water with carbonate hardness is softened by treating with an ion-exchanger to replace the calcium and magnesium ions with sodium ions, causing the water to be alkaline, with pH higher than 8.0, brew liquor color has a black color shade. This is due to formation of thearubigin salts. In tea evaluation, the requirement for water is that it should be uniform. Generally, use of distilled or deionized water is more appropriate.

Temperature of Brewing Water. In tea evaluation, boiling water is generally used for brewing. A temperature near 100°C is considered the standard. Except for selected tender green tea leaf that prefers a lower water temperature of 80–90°C, water that is overboiled or lower temperature is considered inappropriate. Only when boiling water is used to brew tea can the tea aroma be fully developed. More soluble components can be dissolved, showing off completely the aroma, sweetness, bitterness, and astringency, and they provide a more accurate evaluation of tea quality. For general consumption of brewed tea, the purpose is to enjoy the tea leaf and a pot of tea. Therefore, the technique in brewing the tea is demanding so that the good characteristics of the tea can be shown and at the same time hiding the poor characteristics. This kind of tea brewing culture demands specific water temperatures for various tea and is different from standard tea evaluation.

d. Requirements for Sampling of Tea Leaf Sampling of tea leaf consists of taking a minimal sample of tea leaf representative of the characteristics from a lot of raw or processed tea leaf for the purpose of its evaluation or examination. Correct or representative sampling of the characteristics of the whole lot of tea leaf is the first task in assuring the accuracy of the result of evaluation or examination. Because tea leaf is usually not homogeneous, sampling must be very careful so that the sample is a proper representative of the whole lot. Besides, the sample is only 3 grams of tea leaf. Sampling requirements are very strict. Therefore, only properly trained and experienced personnel should conduct the sampling.

2. Procedures and Methods of Examination

a. Brewing Method A sample of 2.83–3.00 grams of tea leaf is weighed out and put into the teacup (as shown in Fig. 7). About 150 mL of boiling water is added to the cup

Figure 7 The standard procedure for the preparation of tea infusion for evaluation is to weigh out 3 grams of tea leaves, pour in 150 mL of boiling water, and infuse for 5 min. The infusion is then poured out for evaluation.

(amount of tea leaf is about 2% of the water used). The cup is covered and let sit for about 5–6 min. The brew liquor is then decanted into the tea bowl for the evaluation of its characteristics. The infused tea leaf is left in the teacup for examination of its aroma.

b. Items to be Evaluated Items to be evaluated generally include outer appearance (shape and color), brew liquor characteristics (color, aroma, and taste) and infused leaf. The standards for each item vary with different teas. Outer appearance is examine for its shape, stringiness, color, white hair at leaf tip, evenness, and foreign matters. Examination of brew liquor color includes its color, clarity, brightness, and turbidity. Brew liquor aroma is examined for its kinds, intensity, strength, clarity, purity, and the presence of off-odors such as rancid, burnt, smoky, greenish, or moldy. Examination of brew liquor taste includes its consistency (thick or thin), sweetness, smoothness, bitterness, liveliness, stimulatory ability, and astringency. Infused tealeaf is examined for its color, leaf spreadability, and integrity of leaf tip. It is also used as a reference to differentiate the cultivars of tea leaf raw materials, maturity of tea leaf, evenness, and degree of fermentation.

c. Evaluation Methods Before brewing, tea leaf is examined for its outer appearance (as show in Fig. 8). After brewing, the infused tea leaf is sniffed three times for the evaluation of its aroma intensity and purity, as well as to detect off-odors such as greenish, smoked, burnt, rancid, and stalled odors and others (as showed in Fig. 9). Then follows the evaluation of brew liquor color for its intensity, clarity, and brightness. When the temperature drops to about 40–42°C, 5–10 mL of brew liquor is put into the mouth and swirled continuously with the tip of the tongue (as shown in Fig. 10). This provides continuous contact of the brew liquor with the taste buds and membranes in the mouth interior lining and thus helps in differentiating its brew liquor texture characteristics such as sweetness, smoothness, bitterness, consistency (thick or thin), activity, stimulatory ability, and astringency. For the taste evaluation of brew liquor, the aroma of brew liquor is expelled through the nose when the tip of the tongue is swirling the brew liquor. Then follows the evaluation of brew

Figure 8 The first step in tea quality evaluation is to observe the appearance of tea leaves and color of the infusion.

liquor aroma. Last is the evaluation of infused tea leaf for its color, nature of the shoot, maturity, evenness and appropriateness of degree of fermentation (13).

B. Quality Characteristics of Partially Fermented Tea

Even though white tea is graded based on its quality and again subgraded into Silver Tip Pekoe, Baimudan, Kungmei, and Shoumei, the manufacture procedures for each are

Figure 9 The second step in tea quality evaluation is to evaluate the aroma of the tea infusion.

Figure 10 The third and last step in tea quality evaluation is to evaluate the flavor (taste) of the infusion.

basically simple and very similar. Mainly, extensive still withering followed by nature drying, or heated dehydration are required to complete the process. Therefore, differences in the general characteristics of white teas are not big, with the common characteristics of "clear brew liquor with white tealeaf." However, there exist extreme differences in quality characteristics for chingcha (Oolong-type tea). This is mainly because of the significant differences in manufacture procedures and steps in the various Oolong-type teas. In addition these procedures and steps are complex. Even though all the chingcha has the general characteristics of "green leaf with red circumference," there exist significant differences in their aroma. For example, Pouchung tea from Taiwan is most demanding for its "natural fragrant flowery aroma" (fresh volatile aroma), whereas Dungding Oolong is most demanding for its "heated tone" and Pomfeng tea is considered the most "gorgeous" tea. As for Tieguanyin tea, besides its characteristic Yin tone, it is known for its tightness, melancholy, and heaviness. Wuyi Yancha is characterized by its lively, sweet, clear, and aromatic tone or the so-called Yan tone or soymilk tone. However, Fenghuan Shuixian is famous for its natural flowery fragrant aroma with rich connotation and repeatable brewing capability (14).

1. Quality Characteristics of White Tea

Manufacture of white tea requires that the fresh tealeaf has "three whites" the bud and the first and second leaf must be covered fully with white hair. In the manufacture of white tea, there is no panning, rolling, shaking or high-temperature drying. Instead, it takes a long time to wither the tea thoroughly, followed by natural drying or low-temperature drying to complete the process. Therefore, quality characteristics of white tea are as its name implies, "whites". White tea is the most demanding as to outer appearance among the six big groups of tea. That is, its outer appearance is a decisive factor in its evaluation. Very few tea groups have such a strict requirement for the outer appearance. In evaluating white tea, there are two decisive factors—leaf tenderness and color. Leaf tenderness requires an abundance of

fine, white hair with a well-developed bud; the color factor requires that the back of tealeaf is silver white, leaf surface grayish green. In other words, good quality white tea meets the criteria of the so-called "silver bud and green leaf with green surface and white back" or sometimes called "clear sky and white ground." A leaf that is grassy green or black, red or yellowish brown is considered of low quality. White tea requires that the aroma be strong and the taste be fresh, pure, and without off-taste. Greenish odor and fermented taste must be avoided.

Due to the harvesting time, area, and cultivar variations, quality of white tea is thus slightly different. White tea from various seasons can be compared: spring tea has the best quality because of its strong aroma, clean taste, pure white hair, and well-developed bud; summer tea has the worst quality due to its greenish bitter taste, skinny bud, and dull color; autumn tea falls between spring and summer tea. The priority of quality among four seasons is similar to that of Oolong tea.

a. Silver Tip Pekoe Tea Silver Tip Pekoe is considered the highest grade white tea. It is believed that it was first developed in 1796 in Fujian Province. Silver Tip Pekoe utilizes only the tender shoot (one bud); in other words, only tender shoots can be used for making Silver Tip pekoe. It requires not only the plucking of tender shoot, but also that the shoot be plucked only on sunny days for good-quality product. Silver Tip Pekoe has the characteristics of needle-shaped bud full of fine, white, silvery hair; a fresh, refreshing aroma; and sweet, smooth, and clean taste.

b. Baimudan Tea Baimudan tea is one grade below Silver Tip Pekoe tea. It is generally manufactured with tea leaf with "one bud and two leaves," and the so-called "green leaf holding the silver needle." Because Baimudan tea has the bud and leaves connected together, and the outer appearance is "green against white" with the shape of a flower, it is called Baimudan (meaning white peony). Its quality characteristics are loose appearance, tea leaf spreading out and covered with fine, white hair; very apparent hairy aroma; clean, sweet, and smooth taste; and bright orange-yellow brew liquor color.

c. Kungmei Tea Kungmei tea is one grade lower than Baimudan tea, but higher than Shoumei tea. Kungmei tea is manufactured from the so-called "one bud and two or three leaves" tender tea leaf and is different from Baimudan tea with its limitation of "one bud and two leaves." Kungmei tea does not utilize tea leaf with banjhi leaves, and the bud is smaller. Therefore, the outer appearance of the product is a smaller bud, grayish green with a shade of yellow in color. Both the aroma and the taste are not as good as that of Baimudan tea.

d. Shoumei Tea Compared with the above-mentioned three kinds of white tea, Shoumei tea does not carry the bud. Shoumei tea is manufactured utilizing the leftover tender tea leaf after the bud has been pulled to manufacture the Silver Tip Pekoe tea. Because the outer appearance of Shoumei tea leaf is similar to the eyebrow of an elderly (long-lived) person, it is called Shoumei (meaning the eyebrow of a long-lived person). The color, aroma, and taste quality of Shoumei tea are lower than Kungmei. It is a lower-grade white tea.

2. Quality Characteristics of Chingcha (Oolong-Type Tea)

The Chingcha (Oolong-type tea) group of tea has many varieties. Due to varietal and procedural differences in processing, considerable quality differences exist among the different Oolong teas. As in the case of white tea, variations in tea leaf plucked at different seasons affect its quality. Spring and winter teas have the best quality, followed by the autumn tea, and then the summer tea, which is poorest, with a more bitter taste and poor aroma. The exception is Pomfeng tea (also called white-haired Oolong tea, champagne

Oolong, or Oriental Beauty tea) produced in Taiwan. It is best manufactured in the summer; where the other seasons' products are of lower quality. Even though the production volume of Oolong tea is not big, but there are a few typical ones: Northern Fujien Chingcha, such as Wuyi Yancha; Southern Fujien Chingcha, such as Anxi Tieguanyin tea; Guangdong Chingcha, such as Fenghuang Shuixian tea; and Taiwanese Chingcha, such as Wenshen Pouchung tea, Dungding Oolong tea, and Pomfeng tea. The major quality difference between white tea and Chingcha is that white tea puts much emphasis on its outer appearance, whereas Chingcha more emphasis on the taste quality of its brew liquor (15).

a. Wuyi Yancha with Its Active, Sweet, Clear, and Aromatic Tone Wuyi Yancha belongs to the Chingcha (Oolong-type tea) group. It was first produced in the Wuyi Mountain, Fujien Province in China at the later period of the Ching Dynasty (1850–1900). Because the tea trees were grown on the cliffs of mountains, thus it is called Yancha (cliff tea).

Wuyi Yancha is one of the historically famous teas. Its characteristics include an even, well-developed outer appearance, tightly curled and stringy, bright and smooth light-green color, long-lasting aroma, and sweet, smooth and soothing taste. It has an active, sweet, clear and aromatic tone, collectively called the Yan tone or soymilk tone. Brew liquor is bright orange yellow. Infused tea leaf shows "green leaf with red circumference."

Quality characteristics of Wuyi Yancha are close to Wenshen Pouchung tea, but with redder brew liquor. In addition, infused tea leaf of Yancha is redder than Anxi Tieguanyin and Guangdong Fenghuang Shuixian teas. Compared with Dungding Oolong, Wuyi Yancha does not require repeated panning and rolling; this provides a more lively and clean flavor (11).

b. The Tight, Melancholy, and Heavy Tieguanyin Tea Tieguanyin tea emphasizes its Guanyin tone. This so-called characteristic Guangyin tone has a long-lasting and highly aromatic tone; it possesses either an orchid aroma (Anxi Tieguanyin) or a weak, fruity, acidic taste (Taiwanese Tieguanyin); and a general sweet and soothing taste. Tieguanyin is the name for the tea itself as well as the name of the tea cultivar. The most famous and best quality Northern Fujien Chingcha is Anxi Tieguanyin. It is considered the superior among all the Southern Fujien Chingcha. It has the same reputation as the Wuyi Yancha of the Norther Fujien Chingcha.

In tea classification, Tieguanyin is considered a partially fermented tea. Its degree of fermentation is slightly higher than Dungding Oolong tea. Like Dungding Oolong tea, it also emphasizes its tone to the throat. The common characteristic of these two kinds of tea is that both require a "repeated panning and mass rolling process." However, Tieguanyin also requires a rolling and baking-in-cloth-bag process. The preliminarily dried tea leaf is wrapped in a square cloth, manually or mechanically mass rolled, followed by a long, slow baking in the cage with low heat to form its characteristic shape and Tieguanyin aroma. This process is only applied in the manufacture of Tieguanyin and not applied in the manufacture of other kinds of tea. In fact, this "rolling-and-baking-in-cloth-bag" process has close similarity with the "roll-in-bag" and "repeated panning and rolling" processes used in the manufacture of Dungding Oolong tea (semispherical Pouchung tea). These typical processes are the key to the formation of characteristic flavors (baked aroma and throat tone). Processing for both Tieguanyin tea and Dungding Oolong tea is labor-intensive, maybe even more so for Tienguanyin tea. Very few manufactured teas require such a long baking treatment as Tieguanyin tea. Fresh tea leaf for Tieguanyin tea passes through a series of complicated manufacturing procedures. It is like a heavily melancholy, middle-aged person going through all the hardships and difficulties in life, experiencing the sweetness, bitterness, sourness, and worries, and has lost all the luster of its nature purity.

The quality characteristics of Tieguanyin tea are as follows: its outer appearance is almost round due to the repeated rolling; its color is a kind of oily black; and it is heavy to handfeel. Its brew liquor has a yellowish brown color; the flavor shows a distinct baking aroma; and the taste is weak fruity-acidic. Most worth mentioning is that, like Dungding Oolong tea, Tieguanyin tea is also considered one of the two types most appropriately brewed in Yixing-type purplish sand tea pots for the so-called elders' tea. These two kinds of tea are characterized by their repeated brewing, with a very satisfactory "throat tone." Drinking Tieguanyin tea is associated with a significant melancholy, heavy, and conservatively tight atmosphere. Its flavor is bitter, sweet, and heavily melancholy, like having difficulty in cheering up (11).

Currently, the main production area for Tieguanyin tea is in Anxi area of the Fujien Province, China, which enjoys the best reputation and has the most abundant. Other production areas have only limited quantity. In Taiwan, is produced only in the Muja area of Taipei City and Shimun area of Taipei County. Because it is very labor-intensive, Tieguanyin is considered one of the high-priced teas.

c. Fenghuang Shuixian Tea with Its High Note, Strong Taste, and Intense Connotation Fenghuang Shuixian tea is manufactured from the tea cultivar with the same name. The most significant characteristic is that the water solubles content in tea can be as high as 45% (dry weight basis), with tea polyphenols over 25%. It is one of the very few cultivars that provide such rich components.

Fenghuang Shuixian tea is produced in the Fenghuang (Phoenix) mountainous area in Guangdong Province, China, with its fresh leaf plucked mainly from the Shuixian cultivar. It is therefore called Fenghuang Shuixian tea. It belongs to the Chingcha (Oolong-type tea) group. Around 1850, it already enjoyed a reputation in the world tea market, and was considered a pearl in the Oolong tea group.

Fenghuang Shuixian tealeaf is solidly stringy, straight, greenish brown in color with an oily luster and a clear elegant aroma with natural flowery aroma. Its brew liquor is clear and brightly yellowish in color, smooth and thick with a sweet aftertaste. The infused tea leaf has a green stem with a green belly and a red circumference. It can be repeatedly brewed (11).

Manufacture procedures for Fenghuang Shuixian tea are similar to those for Wuyi Yancha, but with a lighter degree of fermentation. The tea's most significant characteristics are its natural flowery aroma and that it can be repeatedly brewed.

d. Wenshen Pouchung Tea with Its Aromatic Characteristics Wenshen Pouchung tea is lightly fermented, with a stringy outer appearance and dark green color. Among the six basic tea groups, this is the one most demanding in its aroma quality in the whole world. Wenshen Pouchung tea is produced mainly in Northern Taiwan, with an annual production of 1500 metric tons. It is the least fermented among all the partially fermented teas, with about 8–18% of the catechins in the fresh tea leaf oxidized. Manufacture of Wenshen Pouchung tea is conducted very gently and carefully in terms of its processing techniques. Because most of the components are not oxidized, its flavor is closer to that of green tea, somewhat between green tea and Oolong tea. Typical characteristics of Wenshen Pouchung tea include clear and volatile aroma with distinct flowery aroma, lively, sweet and smooth taste, as well as bright greenish yellow color. In general, its quality characteristic is that it gives an instant lively and stimulatory happy feeling after consumption (9,10).

e. Dungding Oolong Tea with its Demanding Baked Tone Dungding Oolong tea is the most famous Taiwanese tea. At the same time, it catches the attention of consumers worldwide. The degree of fermentation for Dungding Oolong tea is fairly extensive among all the partially fermented teas, but below that for Pomfeng tea. Its annual production is 15,000 metric tons. It is different from Wenshen Pouchung tea in that its shape is

semispherical, thus it is also called semispherical Pouchung tea. It is produced in all the major tea production areas in Taiwan. Due to the superior reputation of Dungding Oolong tea produced in the Luku Shiang of Nantou County in Taiwan, the term Dungding Oolong Tea is used to represent this kind of tea.

Today, Dungding Oolong tea is the major tea produced in Taiwan. Its manufacture procedures are very complicated and labor intensive. Due to the processing techniques, and its more extensive degree of fermentation, with 15–30% of its catechins oxidized, the sensory quality of Dungding Oolong tea shows a more mature and artificial flavor. Typical characteristics of Dungding Oolong tea include a very satisfactory throat tone, very significant baked tone and aroma, and a long-lasting aftertaste. If Wenshen Pouchung tea is considered the most demanding of its aroma in the world, Dungding Oolong tea is the most demanding for its throat tone. Formation of the typical throat tone and baked aroma in Dungding Oolong tea is dependent on its long baking time and reprocessing. Therefore, it is very labor intensive. Tea without the presence of the significant baked aroma and throat tone is not considered a Dungding Oolong tea (9,10).

f. The Most Elegant and Beautiful Pomfeng Tea Pomfeng tea is also called white-haired Oolong tea, Oriental Beauty tea, and Champagne tea. It is most likely the most expensive tea in the world. It is the tea with the highest degree of fermentation within the partially fermented tea group. This tea has the following characteristics. It is strictly and carefully hand-manufactured. It takes at least 4000–5000 tea shoots to make 1 kg as compared to 2000–3000 for the other partially fermented teas. Pomfeng tea is made almost completely from tea leaf with one bud and two leaves. Pomfeng tea is manufactured only in the summer in a few cities and counties in Taiwan. Only leaf from the Chin-shin Dapang cultivar is used in manufacturing of Pomfeng tea. Leaf is hand-plucked and only leaf that had been infested with the tea green fly (*Empousca flavescens* Fabricus) can be manufactured into better quality Pomfeng tea. Based on the above-mentioned criteria, Pomfeng tea is very precious and limited, and is a very special tea produced only in Taiwan, and nowhere else. The annual production is about 50 metric tons, and is the most expensive tea.

Because Pomfeng tea has gone through a fairly extensive fermentation, its quality characteristic is that it does not have the fairly easily detected uncomfortable raw-greenish or off-greenish odor as in other partially fermented tea. Its aroma is typical of the natural ripe fruit aroma and its taste has a honeylike sweet aftertaste tone. In addition, lower temperatures are used in the panning and drying of Pomfeng tea, and baking is strictly forbidden, thus its aroma does not have a significant baked tone. Because Pomfeng tea is manufactured strictly from young tender tea leaf, it is rich in amino acids and consequently provides a brothy and clean mouthfeel. The brew liquor of Pomfeng tea is not bitter or astringent, because about half of the catechins are oxidized in Pomfeng tea. Typical Pomfeng tea has a beautiful outer appearance with significant alternative red, white, yellow, brown, and green colors, is naturally curled liked a flower, and the brew liquor has a bright amber color. Its quality is close to black tea, somewhat between Oolong tea and black tea. The aroma of Pomfeng tea is formed mainly through fermentation and has a more matured flavor. Due to its scarcity, Pomfeng tea can be considered the world's most elegant and beautiful tea. Pomfeng tea was exported to England earlier and was much appreciated by Queen Victoria; thus, it is also called Oriental Beauty Tea (10).

V. PACKAGING AND STORAGE OF PARTIALLY FERMENTED TEA (10)

In tea manufacture—whether of green tea, partially fermented tea, or fully fermented tea—fresh tea leaf is plucked, transported to the factory, and finish-processed with the final step

of drying to the final product. This raw tea or roughly processed tea undergoes change in moisture content: from about 75% (wet weight basis) moisture is rapidly reduced to below 5%. Because raw tea or roughly processed tea go through such a drying process, it is a product with very low moisture and with water activity usually < 0.1. In addition, the drying process is accomplished with a temperature around 100°C, usually for over 1 hr; thus, the enzymes in the fresh tea leaf are either completely inactivated or destroyed. Theoretically, commercial tea is a product with low water activity and no enzymatic activity. It should have very long shelf life. However, tea has a shelf life shorter than most other dried products. The main reason is that tea itself has a great tendency to absorb moisture and other off-flavors. In addition, the flavor of tea is formed mostly from reprocessing. In comparison with the flavor of other habitually consumed beverages like coffee and wine, tea flavor is rather unstable and sensitive. It vaporizes by itself and is re-oxidized, which induces flavor change. Besides the tendency of tea to absorb moisture and off-flavor, harming its quality, oxygen, high temperature, and light are factors that are also responsible for the deterioration of tea quality. Therefore, the shelf life of tea is generally not as long as other dried products.

Even though scientific literature is not readily available on the safety and sanitation problems related to tea due to improper packaging and storage, proper packaging and storage are still important economic factors because tea is a flavor-directed almost addictive food product.

Among all the teas with various degree of fermentation, the ones with higher degree of fermentation have better storage stability. In consequence, black tea has the best storage stability, followed by Oolong tea, Pouchung tea, and white tea. Unfermented green tea has the poorest storage stability for two reasons. The first is that most of the components in unfermented tea have not been oxidized; they continue to oxidize during storage. In contrast, part of the components in fermented tea have undergone oxidation, and the rest are not that easily oxidized during storage. The second reason is that flavor-contributing components are very limited in green tea and comparatively much less than fermented tea, so minor changes in these components in green tea can be detected easily. Therefore, green tea cannot be stored for long periods. Also, spherical shaped tea has better storage stability than stringy type tea, and stringy type tea is more stable than tea in broken pieces. The reason for the poor storage stability of broken piece–type tea is that it has a larger exposed surface area for moisture absorption and oxidation. With drying and baking, the longer the heating processes, the better the storage stability. This is related to the removal of unstable components and the stabilization of some of the components during heating. Tea with light aroma and tea that is considered as upper-grade tea has poor storage stability. This is related to the general instability, natural losses, and oxidation of the tea flavor components. Some of the partially fermented teas, such as Pouchung and Oolong teas, are considered as the most demanding on their aroma. In order to ensure their economic values, it is important to properly package and store the tea, and prevent quality changes during storage.

A. Factors Causing the Deterioration of Tea Quality

Quality changes in tea during storage can be divided into two stages. The initial stage of storage, post-fermentation, also called post-maturation, in fact improves the quality. Subsequent storage will induce quality deterioration due to natural or external factors. In the latter stage of tea storage, even with the advances in modern packaging technology and materials, it is still difficult to prevent quality deterioration completely during storage—only, at most, to delay the deterioration rate. The initiation of post-fermentation and mechanism of post-fermentation are still not completely understood. Many scientific

reports say that some of the astringent polyphenolic components and the proteins in tea are conjugated naturally, thus reducing part of the astringent taste and off-greenish odor and improving the brew liquor flavor. The post-fermentation process is accelerated due to absorption of moisture. Some researchers believe that the post-fermentation process is related to the residual polyphenol oxidase and peroxidase activities that are also accelerated by moisture absorption. Others believe that post-fermentation is related to the auto-oxidation of polyphenols. In any case quality deterioration of tea during storage proceeds slowly, and it is difficult to retain its original fresh quality.

Factors causing quality deterioration in tea storage includes moisture content, light, temperature, oxygen, time, and absorption of off-odor. The effects of these factors are discussed below.

1. Moisture Content in Tea

The safety limit for moisture content in tea is 5%. The protective, monolayer moisture content is about 3–5%, depending on the kind of tea. Theoretically, tea that is too dry is also not suitable for storage, lacking the protective, monolayer moisture content against oxidation. However, tea is a product with high moisture absorption capability. After moisture absorption in tea, reactions of residual polypenol oxidase and peroxidase will be activated. When the moisture content of tea is above 7%, many undesirable reactions will be accelerated. When moisture content is above 12%, tea is vulnerable to mold growth.

2. Light

Among all the factors causing quality deterioration in tea during storage, exposure to light is the most aggressive. This has been shown by many experiments with the same conclusion. Several days to a few weeks of light exposure will show significant quality deterioration in tea, whereas other deterioration factors will take 1–2 months. This quality deterioration is even more serious during prolonged storage. For example, in black tea experiments, samples exposed to light deteriorate much faster than samples exposed to moisture absorption, and its value also decreases significantly. In green tea experiments, tea quality deteriorates significantly after exposure to light for 2 weeks. The same result holds true for Pouchung tea.

Many components in tea are sensitive to light. Catechins themselves are light sensitive. Chlorophyll, when exposed to light, oxidizes and loses its green color. Carotenoids and some flavor-related compounds such as unsaturated fatty acids can conduct oxidative hydrolysis when exposed to light. To this day, exposure to light is considered the factor that caused the most rapid quality deterioration in tea. Even exposure to a weak light (< 50 Lux) can induce quality deterioration in tea. Many foods show the "solar off-odor" after exposure to sunlight. The same holds true for tea. Bovolide is the typical compound formed after exposing tea to sunlight. Examination for this compound can be used to judge whether the tea sample has been exposed to sunlight. Because light is the most influential factor on quality deterioration in tea, adequate prevention from exposure to sunlight and use of sunlight-proof packaging materials for proper storage of tea are absolutely necessary.

3. Temperature

High temperature accelerates many chemical reactions, causing deterioration in tea quality. Therefore, the higher the storage temperature, the more rapid is the deterioration. For green tea, it is very difficult to retain the green color after high-temperature storage, and the brew liquor color also turns brown rapidly. For tea with fragrant aroma, high temperature

accelerates the disappearance of the sensitive, volatile flavoring components. Experiments to date demonstrate that low-temperature storage is the most direct and effective method. Together with other moisture and oxygen-preventive measures, low temperature is the only method to maintain the original freshness and flavor in tea.

4. Oxygen

Tea contains many compounds that can be oxidized and subsequently deteriorate tea quality during storage. For example, catechins oxidize and induce deterioration in taste and color of the brew liquor. Ascorbic acid oxidizes and subsequently reacts with amino acids to form brown-colored compounds. Some flavor-related unsaturated fatty acids oxidize and form volatile aldehydes and alcohols with the formation of aged and rancid taste. Tea quality will deteriorate gradually during storage if teas are not protected from the occurrence of oxidation.

5. Time

Theoretically, the longer the storage period, the more unstable is the tea quality, with the exception of "aged tea." The formation of aged, rancid, and acidic tastes in tea during storage is closely related to the storage time. Many intrinsic and extrinsic factors generally contribute to the deterioration of tea quality with time. Good packaging and storage methods can only delay the deterioration of the quality. Under conditions of high temperature and relative humidity, its takes only a short time to induce deterioration in tea quality.

6. Absorption of Off-Odor

The fine structure in tea leaf itself is porous. There are many observable capillary structures on the tea leaf surface and interior. External air and moisture can easily be adsorbed on the tea surface through physical phenomena. In addition, tea leaf itself has many polar and nonpolar compounds such as sugars, polyphenols, and fatty acids. These compounds have a strong affinity to the polar and nonpolar organic compounds in air. Therefore, tea can easily absorb the off-odors from air. Because tea is an almost addictive product very sensitive to aroma, very minor changes in its composition or formation of off-taste will induce considerable loss of its economic value.

B. Preventive Methods for Deterioration of Tea Quality

Ancestors in China recognized long time ago the five avoidable principles in preserving tea quality: moisture content too high, overexposure to the open environment, exposure to light, high temperature and off-odors. These five avoidable principles fully describe the critical points to follow in preserving tea quality. Even with the modern scientific studies on packaging and storage of tea, these guidelines are still being followed.

1. Avoidance of Too-High Moisture in Tea Leaf

The first criterion in preserving quality of tea leaf is to control its moisture content. If the moisture content of tea leaf cannot be controlled within the safety range of 3–5%, no better packaging methods can prevent the deterioration of tea leaf quality. Raw tea leaf with one-step drying usually cannot reach the fully dehydrated status; application of two-steps drying procedure is a safer operation. Tea leaf right after drying has a high temperature and requires a cooling-off standing process before it can be bagged. This cooling period should

not be conducted too long in order to avoid reabsorption of moisture and off-odors. Processed tea leaf after removal of stems, broken pieces, and powders should be redried to assure that the moisture content is below 5%.

2. Avoidance of Exposure to Light

Exposure of tea leaf to light (including radiant or sunlight) can induce drastic deterioration in its quality due to the many components in tea leaf that are vulnerable to photochemical reactions. Therefore, tea leaf from finishing to packaging should be protected from exposure to light, especially in conducting the removal of stems, broken pieces, and powder directly under sunlight (including ultraviolet light). Transparent polyethylene (PE) bags are not suitable containers for finished tea leaf because of the difficulty in shielding the product from exposure to light. From the standpoint of modern packaging materials, the commonly used laminated aluminum foils and composite paper cans are good packaging materials, blocking light effectively. It is therefore not difficult to prevent exposure of tea leaf to light through proper packaging.

3. Utilization of Low-Temperature Storage

Up to this time, low-temperature storage is recognized as the most effective means of maintaining tea leaf quality. Tea color, aroma, and taste quality can be maintained at its best by low-temperature storage. Low-temperature storage of tea leaf is an excellent method for maintaining its freshness and avoiding the development of aged and rancid flavor, as well as color and appearance changes.

Theoretically, the lower the temperature, the better the keep quality. Storage of tea leaf at $-20^{\circ}C$ can maintain its quality almost unchanged for long period of time. However, from the economic standpoint, a storage temperature of 0–5°C is a more appropriate. During low-temperature storage, tea leaf still absorbs moisture. Maintenance of relative humidity at 50–60% in the refrigerator is also critical. Avoidance of off-odor during refrigerated storage is worth noting. It is better to have a ventilated, refrigated system.

4. Utilization of Oxygen-Free Packaging

Oxygen is present everywhere, with about 20% in concentration in air. Presence of air is definitely related to deterioration of tea quality during storage, giving rise to "aged," rancid, and reversed taste, discoloration, and weakening of the lively taste. Solution to the problem of oxygen in packaged containers is urgently needed. There are many methods to prevent the tealeaf from reoxidization during storage, such as vacuum packaging or nitrogen-flush packaging. These two techniques are employed commonly in packaging of various kinds of food, including tea, to prevent the re-occurrence of oxidation during storage. For example, sencha (Japanese green tea) in Japan is exclusively packed and stored in nitrogen. Many studies have proved that vacuum packaging or nitrogen-flush packaging can effectively preserve the color, aroma, and taste quality of tea. In combination with refrigerated storage, this is the best method known to preserve tea leaf quality. Besides using vacuum packaging and nitrogen flush packaging, the utilization of oxygen absorbents or scavengers is similarly effective. This procedure can effectively preserve the color, aroma, and taste quality of sencha for 7 months. At the same time, there is only a small loss of ascorbic acid. As compared to tea leaf packaged with presence of oxygen, quality deterioration of the latter is more significantly. Application of oxygen absorbents or scavengers can effectively reduce the quality deterioration of partially fermented tea such as Pouchung tea during storage.

C. Packaging Materials and Forms for Tea

Storage and packaging of tea are two sides of the same coin. Good storage stability depends on proper packaging to achieve. Packaging of tea leaf basically has to meet the following criteria: material with low permeability to moisture and air, odorless, opaque to light, and compressable. Besides these criteria, there are also considerations of production cost and attractiveness. With advances in packaging materials, it is not difficult to meet all these criteria. However, the most expensive packaging material may not be the most suitable one for packaging tea leaf. The commonly used packaging material and forms are discussed briefly below.

1. Polyethylene (PE) or Plastic Bags

PE bag is a fairly commonly used package for tea leaf in Taiwan. In general, there are two kinds, the low-density and high-density PE. Low-density PE bags are more permeable to moisture and air than the high-density PE. The advantages of PE is that it is economical, with reasonable resistance to moisture, and its disadvantages are transparency and permeability to air. So it is not suitable to be used alone for packaging tea leaf. Currently, many tea leaf wholesalers still use PE bags for direct packaging of tea. This is not suitable for maintaining quality during storage: it is better to have another package that is opaque and more resistant to air permeability, such as composite paper cans and metallic cans. Because PE bags are fairly permeable to air, the problem of air inside the package is not solved. Therefore, PE is not a very desirable packaging material.

2. Metal Cans

Advantages of metal cans include resistance to compression and good quality feeling. It is used as the exterior package for tea leaf. It was common in earlier tea leaf packages to use PE bags inside with metal cans outside. However, it is more expensive, and cans are easily corroded and get rusty. This has now gradually been replaced by other packaging. Metal cans cannot be used directly to pack tea leaf. This is because of the odor from the lacquer to coat the metal and poor tightness of seal in these cans. High quality is lost when tea leaf is packaged exclusively in metal cans. Currently, in Taiwan, use of composite paper cans has gradually replaced metal cans.

3. Composite-Paper Cans

Composite-paper cans are used most commonly in Taiwan for packaging. Advantages are economy and light and beautiful appearance; disadvantages are high permeability to moisture and air. If used independently for packaging tea leaf, composite-paper cans have the problem of moisture absorption and permeability to air. They are best accompanied by use of PE bags or aluminum laminated bags as interior packages. This combination of two packages provides resistance to moisture, air, and light and absence of oxygen (use of vacuum, nitrogen-flush, or oxygen absorbent or scavenger), and solves all the problems simultaneously.

4. Aluminum Laminated Pouches

Aluminum laminated pouches have a beautiful outer appearance and the advantage of exceptional impermeability to moisture, light, and air. However, they are more expensive than PE bags. Because of compression problems if used alone, these packages are often

equipped with a cardboard box or composite-paper box as an exterior package. Aluminum laminated pouches can be used with vacuum packaging, nitrogen-flush packaging, or oxygen absorbents or scavengers, and solve the oxygen problem in the bag. In combination with cardboard or corrugated paper boxes aluminum laminated pouches provide a perfect solution in preventing damage from moisture, air, light, and oxygen. When used with low-temperature storage, these packages are the most desirable preservation method for tea leaf. Aluminum laminated pouches have PE, oriented polypropylene (OPP), or casting polypropylene (CPP) on the most interior and exterior layers. They are very resistant to air permeability.

5. Glass Jars, China or Ceramic Jars, Wooden Boxes, and Paper Cartons

Theoretically, these are not desirable packages for tea leaf. Glass is transparent to light and is fragile, heavy, and expensive. Even though glass is very resistant to moisture, it is not completely desirable. China or ceramic jars are similar to glass jars; they prevent light but are fragile and heavy. Wooden boxes, when treated improperly, can easily cause the tea leaf to absorb its off-odor. Wood also absorbs moisture and is permeable to air easily. Paper boxes and bags absorb moisture easily and also are fragile. They cannot be used independently. None of these materials is suitable for packaging tea.

6. Nitrogen Flush or Vacuum Packaging

Because vacuum packaging of tea leaf shrinks the bags, it is more desirable to have an outer package to protect the tea leaf from being squeezed and bumped, and at the same time, provide a beautiful appearance. Packages with nitrogen flush appear to bulge. To avoid explosion, they should not be squeezed. Both of these packages require an outer package. Theoretically, both have similar, desirable effects on preservation of tea leaf.

D. Labeling of Tea Leaf Packages

Labeling requirements for tea leaf in Taiwan are not strict. This is mainly due to the various kinds of tea produced in Taiwan that are used for local consumption and for marketing. Correct and clear labeling of kinds of tea, manufacture date, location of production, manufacturer, brewing and consumption methods can help establishment of brand names and marketing. There is absence of strict requirements for shelf life. Some indicate 1 or 2 years. Theoretically, a clear message about consuming the tea as soon as possible after opening the package is a more desirable arrangement.

VI. DIVERSIFIED UTILIZATION FOR TEA (10)

Tea is a natural, healthy beverage. This is proved by recent scientific studies. Tea is brewed and then consumed. This seems a commonsense method to consumers and is the most common form of tea consumption nowadays. However, utilization of tea leaf for soup or food-dish preparation, and even in common items in our daily lives, is not that well known. With progress in our society, advances in science and technology, and elevation of living standards, people nowadays are more concerned about the food they consume, with emphasis on three requirements—nutritional value, sensory qualities, and functional properties. In order to meet this consumption trend, various food-processing technologies have diversified, and tea is no exception. In recent years, all the tea-producing countries are

aggressively developing diversified utilization for tea in order to elevate its economic value, improve the health status of consumers through their daily diets, and increase the competitiveness of tea in the beverage industry.

Historically, diversified development and utilization of tea has been in existence since the old days. According to written records, the development and utilization of tea started as one of the items used in offerings to spirits, then as a food preparation ingredient, medicine, and eventually as a beverage. In the manufacture of tea, fresh tea leaf generally is plucked at the stage of "one bud and two or three leaves" even though older leaf below the third leaf is also plucked. In fact, various parts of a tea plant can be used. This includes the tender leaves, older leaves, trunk, root, flower, and seeds. The utilized forms include fresh tea leaf, finished tea leaf, tea powder, brew liquor, decolorized tea liquid, and infused tea leaf. With these raw tea materials and the application of scientific formulations and appropriate processing, various tea-based products have been developed and are available, as shown in Fig. 11.

Based on the level of processing, diversified tea-based products can be divided into three categories:

Utilization of preliminary processed tea. This utilizes the preliminary processed tea such as green tea, Pouchung tea, Oolong tea, and black tea as the raw material. It applies the basic and simple food-processing techniques, and at the same time preserves the original tea flavor, in the development of various flavored and conveniently consumed products such as flavored tea, scented tea, and tea bags.

Applications of reprocessed tea. This applies more advanced processing techniques such as grinding, extraction, concentration, can sealing, sterilization, and compression in the reprocessing of raw tea materials. The tea is processed into products such as tea powder, tea jelly, tea candy, bottled or canned tea beverages, instant tea, fruit tea, tea wine, tea champagne, tea pastries, tea pillows, and tea bath bags.

Utilization of tea extracts. This utilizes the fresh tea leaf, as well as secondary grade and off-grade tea as the raw materials. The functional components are extracted

Figure 11 Various diversified tea products.

with hot water or organic solvents, separated, and purified as natural antioxidants, deodorants, and health supplements.

Tea has its own characteristic aroma and taste. It also contains various functional components and inorganic nutrients. When used as a food ingredient, tea reduces oily sensation and fish odor, has a clean sensation, and improves color and taste. Tea itself has antibacterial, antioxidative, and heavy metal–neutralizing effects, as well as other health maintenance functions. Therefore, tea can be utilized to formulate various diversified products and has extreme development potential. When utilized properly, preserving its characteristics and nature and applying the principles of scientific formulation and proper processing, tea can be formulated into various tea products with different color, aroma, and taste. Fig. 12 presents some of the diversified tea products.

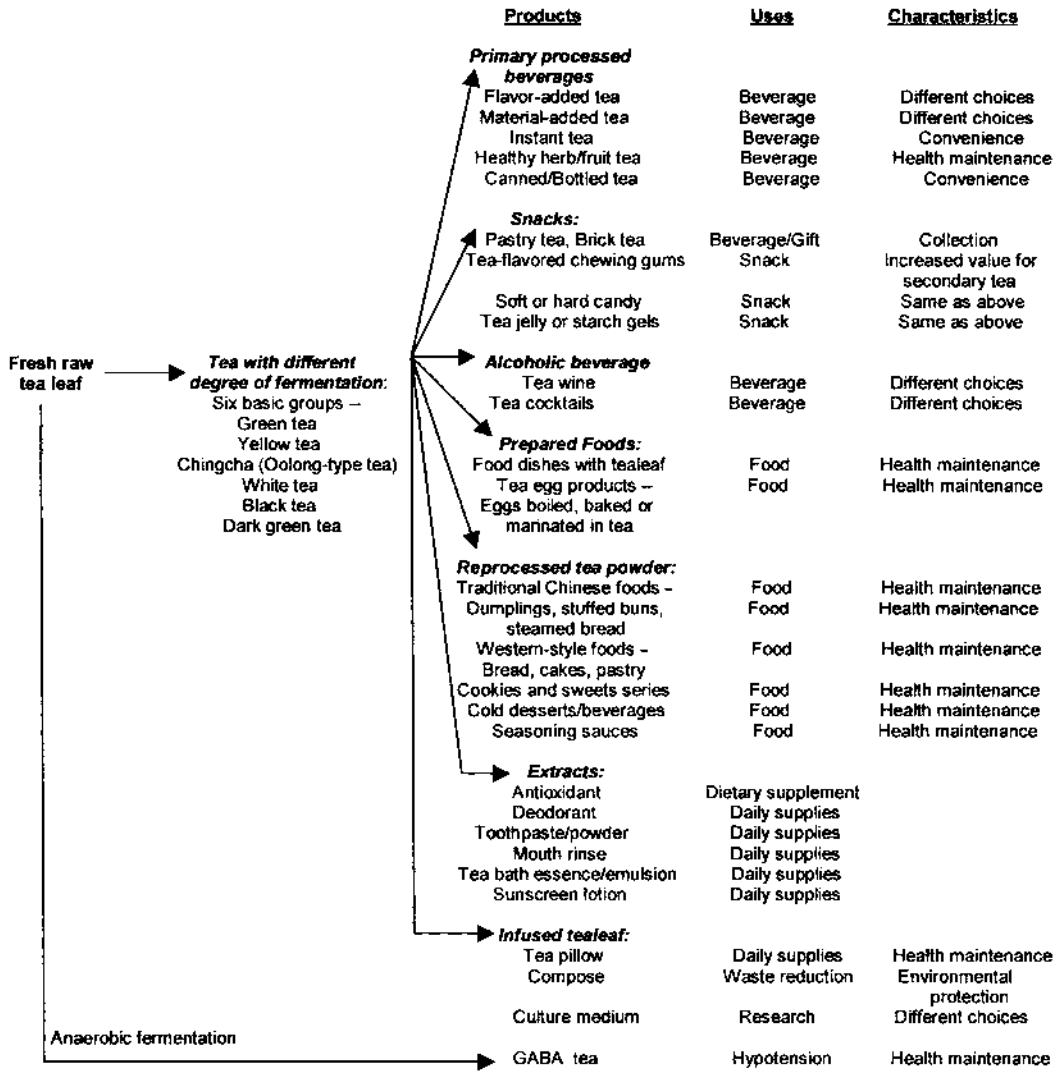

Figure 12 Products, uses, and characteristics of tea.

A. Development of Diversified Tea Products

1. Flavored Tea

Flavored tea is typical of tea diversification and varieties. Flavored tea is a general term covering the different products such as flavor-enriched tea, component-enriched tea, flavored tea, and herb tea. It is manufactured mainly by addition of edible flower, fruit, and stem from horticultural or medicinal plants with special flavors or by spraying its juice or essence through a sprayer onto the tea leaf, thus creating beverages with tea and special fruit/flower aroma. Examples of flavored tea include lemon-flavored black tea, rose-flavored black tea, Roselle (*Hibiscus sabdariffa* L.) black tea, peppermint black tea, jasmine tea, ginseng green tea, and others.

2. Caky Tea and Brick Tea

Caky tea and brick tea are very old processed tea products. They appeared in the early literature. Their manufacture processes are very similar. Basically, tea leaf is cut into 0.5–1 cm strips or not even cut. They are steamed to soften or heated to fluff. They are put in a fixed mold and pressed manually or with a hydraulic press to form a shape. When cooled, it is taken out from the mold and dried into caky tea or brick tea. Tea from these cake and brick are used for regular consumption, or the tea can be made into different shapes as gifts for ornamental purpose. For long-term storage and viewing, caky tea and brick tea should be shrink wrapped/packaged with PVC plastic film.

3. Citrus Fruit Tea

Fruit tea utilizes pomelo or sour mandarin orange with tea leaf added to it, and manufactured into a very special tea. This kind of tea has been popular in the Hakka communities in Taiwan for a long time. The outer appearance of this tea is a flat circular block with pitch-black color and tied with red or white string. For consumption, the blocks are cut into pieces and brewed. It has a special flavor.

4. Tea Bags

Tea bags are convenient, without the disposal problem of loose infused tea leaf. The flavor is similar to the tea brewed from a teapot. It meets the requirement of modern living and office environment. Therefore, its development potential is very good. The manufacture of tea bags applies the modern fixed-quantity packaging technique. The small pieces of tea leaf are packed at a uniform weight (2.0–2.2 g per bag) in filter paper (sometimes called long-fiber paper). Because the tea leaf is in small pieces, it shortens the time for brewing.

5. Instant Tea

Instant tea is a convenient tea product manufactured by extracting, concentrating, and dehydrating the water-solubles from tea leaf into powder or aggregates. It has not only the original tea flavor but also convenience in brewing in either cold or hot water. It can also be put into automatic vending machines for marketing. It meets the demand of modern lifestyles.

6. Canned Tea Beverages

Because of its convenience, time savings, and various features that fulfill modern consumption demands, canned tea beverage has been the new favorite in the beverage market

for the past 20 years. Canned tea beverages are made by extracting the tea water-solubles and processing them into different products. Currently, all the different tea groups have been successfully manufactured into canned beverage forms. Therefore, consumers have various choices.

7. Tea Jelly, Tea Liquor Gels, and Tea Gels

Tea jelly is a jelly-type product made from brewed tea liquor. Tea liquor gel is similar to tea jelly except that small red bean (azuki bean) paste is added before gelation. Tea gel is a high-calorie snack, with less water in the formulation of tea liquor gel and therefore easier to preserve. Tea jelly has the advantages of using natural colorants from the tea leaf itself, and its product has a clear, transparent, and bright appearances with different shapes as preferred by the manufacturers. Its also offers wide taste choices, such as Roselle black tea jelly, Wenshen Pouchung tea jelly, black tea jelly, Oolong tea jelly, and others. Tea jelly contains very little sugar and utilizes nonabsorbable hydrocolloids as coagulants. It gives the stomach a full sensation. It is a suitable low-calorie food.

8. Tea Candy

Most consumers like candy. Candy made from formulations with various tea and fruit flavors has a characteristic tea flavor, and its taste intensifies with longer chewing or sucking. The raw materials and procedures in making tea candy are similar to that of making other candies except that tea leaf or tea extract is included in the formulation.

9. Alcoholic Tea Beverages and Cocktails

Tea and alcoholic beverages have a similar status in human culture. They both color and lighten human lives, and also smooth communications. Tea is considered a warming beverage, and acohol is stimulatory. They belong to two different categories; each possess its own characteristics. Combining the softness of tea and the strength of alcoholic beverages can formulate alcoholic tea beverages and cocktails with special effects.

10. Food Dishes with Tea Leaf

One of the early ways of utilizing tea is cooking it before consumption. However, tea can be used in various tea preparations. Healthy and fine food dishes can be prepared by utilizing the tea leaf, brew liquor, infused tea leaf, or tea powder from different tea groups such as Oolong tea, Wenshen tea, Tieguanyin tea, Pomfeng tea, and black tea in combination with poultry, red meat, seafood, and leafy green vegetables.

11. Tea Eggs

Egg is very nutritious but has its typical egg flavor. It has a short shelf life and deteriorates easily. However, eggs can be processed in conjunction with tea into stable products with improved flavor and mouthfeel. These tea egg products possess the high nutritive values of egg and tea. Eggs are hard-boiled with tea and a few herbs to make tea leaf eggs. Eggs can also be baked in tea leaf in the baking oven until the egg white changes to a brown color while the white color of egg shell. Baked tea leaf egg has a typical tea flavor and the egg yolk is not oily to the throat. Eggs soaked in alcoholic tea beverages for long period can be boiled or baked before consumption. These eggs not only possess the typical flavor but are also shelf-stable.

12. Powdered Tea

Tea contains many components beneficial to health. It is unfortunate that consumption of only the brew liquor does not completely utilize all these beneficial components. However, consumption of the tea leaf directly can provide rich nutrients such as vitamins, minerals, and dietary fiber, and at the same time has the beneficial effect of brewed tea liquor. Powdered tea is made from the freshly plucked tea leaf. After simple processing, dried tea leaf is then ground into an even-particle-sized and colored powder. Because tea powder contains all the components in tea leaf, the human body can utilize all these healthful components when tea powder is consumed. It has also uniform quality and composition. Tea powder can be brewed, or used in flavoring various food formulations, as a food colorant or flavor modifier.

13. Tea Pastry

Tea pastry combines the consumption of rice or wheat with tea, and by developing new products containing these ingredients, tea is part of the daily diet. Fresh tea leaves after blanching and grinding, brewed tea liquor, or tea powder all can be applied directly to the raw materials such as rice or wheat flour in the making of traditional tea pastry. Examples of tea-containing pastry are raised flour cake, bowled rice cake, steamed rice cake, dumplings, vegetable-stuffed cake (steamed), bread (baked or steamed), noodles, and others.

14. GABA Tea

GABA tea is a new type of tea with health benefits, manufactured in anaerobic condition. GABA tea is so called because it contains high concentration of γ-aminobutyric acid (GABA). In animal and human studies, γ-aminobutyric acid has a hypotensive effect; another metabolite in anaerobic fermentation of GABA tea is alanine, which has an alcohol-detoxifying effect. GABA tea is truly a natural healthy beverage (16–18).

B. Development of Tea Culture Activities, Tourism, and the Recreational Tea Industry

In the diversified utilization of tea leaf, besides the development of multi-faced products, tea is different from other agricultural products or foods. Tea leaf also plays an important role in the dietetic culture. Combination of tea drinking and the arts and humanities forms a characteristic tea culture. This cultural activity starts with the visible tea utensils, environment, and surrounding hardware equipment and extends to the invisible human relationship, manners, life nourishment, and even the development of literature, poetry, and songs. Whether development of visible utensils related to tea consumption or development of invisible tea manners, they all have a significant effect on mentoring of the tea consumption. Besides, development of recreational tourism in tea farms is also a very important area in diversification and development of the tea industry. Example of these activities and participation include learning about the growth of tea trees, the biological environment of tea farms, and soil and water conservation; citizen's tea farm; self-assisted tea manufacture; and classrooms for tea art/ceremony. These developments elevate the tea industry from a preliminary level industry to a secondary and then to the tertiary level industry, and help to sustain the tea industry.

VII. HEALTH BENEFITS OF TEA (10)

Tea has been considered a natural, healthy drink worldwide. There are countless written records or myths of tea's healthfulness throughout history. The three most famous ones in Asia are as follows. First, there is the written record of a myth that Sun Nong, the Chinese God of medicine, was tasting the various herbs for their toxicity and was poisoned 72 times, but he was fortunate to get tea to detoxify or neutralize these toxins. The second written record is on the *Appendix to the Materia Medica* (Source of Herbs) (Ben Cao Shi Yi) written by the famous pharmacologist Jongchi Chan in the Tang dynasty in China (about 740 A.D.). It says, "Various medications are for the cure of different diseases, but tea is the medication for all diseases." It implies that tea to a certain extent has many health maintenance functions. The third record is the book *Life Maintenance by Consuming Tea* written by the tea founder and Buddhist priest Eisai in Japan (1145–1215 A.D.). He says at the beginning of his book: "Tea is a health maintenance herb from the Gods, it also extends one's lifespan." Even though the recognition of tea's health maintenance function came from accumulated experiences and myths in the old days, modern scientific investigations have proved its effects on health maintenance with over 1000 research articles in the past 20–30 years. The antioxidative, antibacterial, and anticarcinogenic functions of tea are significant in health maintenance.

A. Characteristic Components in Tea and Their Physiological Functions

The characteristic components in tea and their physiological functions are summarized as followed. Table 2.

1. Catechins

Catechins are also commonly called tea tannins. These polyphenols are typical tea components. Their chemical structures and physiological functions are completely different from the other tannins or tannic acid.

In tea, catechins make up about 10–30% of the dry weight and about 40–50% of the water-solubles. They are the main components in tea liquor. They provide the bitter and astringent tastes and affect the taste of brew liquor the most. Catechins combine with the caffeine in tea and reduce caffeine's physiological functions in humans. Through animal studies and in vivo experiments, researchers worldwide have concluded that catechins and their oxidized polymers (compounds formed during tea fermentation) possess the following functions: antioxidation, antimutagenesis, anticarcinogenesis, lowering of cholesterol and low-density lipoproteins in blood, inhibition on increase in blood pressure, inhibition of platelet aggregation, and antibacterial and anti-allergenic functions.

2. Caffeine

About 2–4% of the dry matter and 8–10% of the water-solubles in tea is caffeine. In tea brewing, about 80% of this caffeine dissolves in the brew liquor. It has a bitter taste and is an important constituent in the taste of brew liquor. In black tea, caffeine combines with polyphenols to form complexes. When the brew liquor cools, these complexes form suspending aggregates and induce tea cream in the cooled tea. This phenomenon is generally called creaming down of tea brew liquor, and is typical for black tea of rich taste and excellent quality. The caffeine in tea and in coffee has the same chemical structure and physiological properties. However, tea has catechins and their oxidized polymers, which can

Table 2 Chemical Components in Tea and Their Physiological Functions

Components	Contents (D.W. basis)	Physiological functions
Catechins and their oxidative condensed compounds	10–30%	Antoxidative, antimutagenic, cancer prevention, lowering of cholesterol, lowering of blood low-density-protein, inhibition of blood pressure elevation, inhibition of blood glucose elevation, inhibition of coagulation of blood platelet, antibacterial, anti-food allergenic, improvement of intestinal microflora, elimination of breath odor
Flavonols	0.6–0.7%	Strengthening of micro-blood vessels, antioxidative, lowering of blood pressure, elimination of breath odor
Caffeine	2–4%	Stimulation of central nerve system, cheering up, heart strengthening, diuretic, anti-asthmatic, stimulated metabolism
Hetero-polysaccharides	ca. 0.6%	Anti-diabetic (inhibit increase in blood glucose)
Vitamin C	150–250 mg%	Anti-scurvy, antioxidative, cancer prevention
Vitamin E	25–70 mg%	Antioxidative, cancer prevention, prevention of sterility
Carotene	13–29 mg%	Antioxidative, cancer prevention, boosting immune system
Saponins	ca. 0.1%	(Cancer prevention, anti-inflammatory effect)
Fluorine	90–350 ppm	Prevention of tooth decay
Zinc	30–75 ppm	Prevention of abnormal taste sensation, prevention of dermatitis, prevention of lowered immune capability
Selenium	1.0–1.8 ppm	Antioxidative, cancer prevention, prevention of cardio-muscle blockage
Magnesium	400–2000 ppm	Antioxidative, enzyme cofactor, increase of immune capability

Source: Refs. 19 through 25.

reduce the metabolic rate of caffeine. Thus, caffeine in tea has a much milder physiological effect on consumers as compared to caffeine in coffee.

3. Minerals

Mineral make up about 5–6% of the dry matter and 60–70% of the hot water-solubles in tea. Tea is rich in potassium, calcium, phosphorus, magnesium, manganese, aluminum, fluoride, copper, zinc, iodine, and selenium.

Tea has more cations and fewer anions. Therefore, tea is considered an alkaline food (pH of the ash is 9.4) that helps to keep the body fluid slightly alkaline and maintain health status. The rich potassium in tea helps the excretion of blood sodium. High blood sodium is one of the inductive factors for hypertension. Potassium dissolves easily in hot water. This is

the reason for tea's antihypertensive effect. Fluoride is recognized by dentists worldwide as having an anticavity effect and is a needed micronutrient in teeth maintenance. Tea has an extremely high level of fluoride and this element is slightly soluble in hot water. The fluoride concentration in brew liquor is about 1 ppm, similar to the amount in potable water with added fluoride. Therefore, many reports proved that tea drinking is very beneficial in the prevention of cavities in school children. Tea is also rich in manganese, one of the needed micronutrients in human. It is very difficult for people to absorb manganese from food. Even though tea is rich in manganese, it is not that soluble in hot water. Therefore, it is not easy to take in manganese from drinking tea. However, Japanese Matcha (powdered tea) and Taiwanese tea powder are good sources for manganese.

4. Vitamins

Tea leaf (especially green tea and Pouchung tea) is rich in carotenoids that can be converted into vitamin A. In addition, tea leaf is rich in vitamins B_1, B_2, C, E, and niacin.

During fermentation, carotenoids in fresh tea leaf are gradually oxidized and decomposed into smaller molecules that serve as the precursors for the flavoring components in these teas. The carotenoids content in tea leaf is about 0.017–0.018% on a dry weight basis. Unfermented green tea has about 0.016% carotenoids, and fully fermented black tea has only 0.007–0.009% carotenoids.

The contents of vitamins B and C in tea leaf are not less than that in fruits and vegetables. These vitamins are water-soluble. Therefore, tea drinking (especially green tea and Pouchung tea) can supplement the intake of these vitamins.

5. Other Functional Components

Tea also contains other functional components such as flavonols (about 0.6–0.7% on dry weight basis) that can strengthen the walls of blood vessels and remove offensive breath odor from the mouth (halitosis). Manufacturers in Japan already apply tea extracts in candies that can remove bad breath odors and market these products. The saponin in tea leaf is about 0.1% (dry weight basis). These compounds were claimed to have anticarcinogenic and anti-inflammatory effects, but these claims have yet to be proved. Recently, a hetero-polysaccharide composed of pentoses and hexoses with amino groups has been isolated from crude aged tea leaf in Japan. This compound has been proven, in animal studies, to have a blood-glucose-lowering effect. The γ-aminobutyric acid in tea can lower blood pressure. In Japan, there are tea manufacturers that aggressively promote the forced anaerobic fermentation of tea to produce large amount of γ-aminobutyric acid in their products. These products are marketed as GABA tea with claims for prevention of hypertension (16–18).

Tea has long been considered a mild health-maintenance beverage. Even though some of these health-maintenance or functional components are gradually being discovered, unveiling its mystery, it is believed that tea leaf still contains many undiscovered functional components. With the advances in scientific studies, they will be gradually discovered.

B. Health Benefits of Tea Leaf

With advances in science and technology, scientists are trying very hard to unveil the physiological effects of this natural health-maintaining beverage—tea. Through various animal or clinical studies, it has been proved that tea leaf truly possesses several health-

maintenance and disease-prevention benefits. The health-maintenance benefits of tea are summarized from various published reports worldwide as follows:

1. Blood-Lipid Lowering Effect and Prevention of Cardiovascular Diseases

When the blood lipid levels are high, cholesterol tends to precipitate on the inner walls of the blood vessels and induce atherosclerosis and blood clots. Tea drinking can lower the level of blood lipids; in particular, the EGCG (epigallocatechin gallate) can lower the low-density lipoproteins (LDL) and increase the high-density lipoproteins (HDL). In rat feeding studies, 0.5–1.0% EGCG in the rat feed can significantly lower the contents of total cholesterol, free cholesterol, low-density lipoproteins, and triglycerides, and increase the high-density lipoprotein content; there are also increases in the cholesterol and lipid contents excreted into the feces.

Tea drinking also reduces blood viscosity and thus prevents the formation of blood clots. Catechins can increase blood coagulation time in rabbits, with an increase in the metabolites of fibrous proteins. These results explain the anticoagulant functions of tea leaf and its role in the prevention of cardiovascular diseases.

2. Prevention of Hypertension

Hypertension is a common disease in older people. High blood sodium levels is one of the reasons. The potassium in tea can induce the excretion of sodium. Potassium is easily soluble in hot water; therefore, tea drinking has a preventive effect against hypertension. Catechins can inhibit the angiotensin converting enzyme activity, and this subsequently proves that tea is an important factor in lowering blood pressure.

3. Lowering of Blood Glucose Levels and Prevention of Diabetes

The EGCG catechin and the hetero-polysaccharide in tea can have significant effect in lowering blood glucose. Recently, in Japan, scientists extracted a water-soluble hetero-polysaccharide and used this compound in a clinical study of 100 patients with diabetes. Results showed significant decreases in blood glucose and urinary glucose in these patients.

4. Prevention of Caries

Tea leaf is rich in fluorides, in particular the older tea leaf. Tea leaf can prevent cavities because fluoride can replace the hydroxyl group of the hydroxyapatite and form the fluorapatite that is more resistant to acid corrosion, thus achieving the anticaries effect. The polyphenols in tea leaf also have a strong bactericidal effect on *Streptococcus mutans* and inhibits the formation of insoluble glucan. These factors together have a comprehensive anticaries effect.

5. Bactericidal and Antiviral Effects

Catechins in tea leaf have bactericidal and inhibitory effects on human pathogens such as *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *V. fluvialis*, *B. cereus*, *C. perfringens*, and *Clostridium botulinum*. Tea leaf can also improve the intestinal microflora by promoting the growth of beneficial microorganisms; also, it has bactericidal and inhibitory effects on some harmful intestinal microorganisms. Green tea is extensively used in the clinical treatment of intestinal dysentery in the former USSR. After consumption of green tea for 2–3 days, the dysentery bacteria are inhibited, and patients may recover completely in 5–10 days.

Besides its effects on human pathogens, tea leaf has also a strong inhibitory effect on many of the pathogenic fungi on human skin. On culture agar, 1.25% tea extract can completely inhibit the growth of ringworm and other skin diseases.

6. Antimutagenesis and Anticarcinogenesis

Cancer is a disease with an extremely high mortality rate. Even though there are many hypotheses on its causes, it boils down to the formation of mutated cells initiated by the various cancer-inducing factors, and because of further promotion by the intrinsic and extrinsic factors, these mutated cells are changed to precancer cells, and eventually develop into cancer cells. When a normal cell is changed to a cancer cell, it has to go through the initiation and promotion stages. Any compound that can inhibit the initiation or promotion can be viewed as having anticarcinogenesic properties. The effective components in tea leaf have inhibitory activities not only on the initiation but also on the promotion of cancer cell development.

In an epidemiological survey in Shizuoka-kan (Prefecture) of Japan, a negative correlation exists between tea drinking and stomach cancer. Numerous in vivo studies on mouse or rats were conducted in many countries on the prevention of various cancers with tea extracts or the effective components in tea (such as catechin-type compounds) and their inhibitory effects. Results indicate that tea to a certain degree has preventive and inhibitory effects on stomach cancer, lung cancer, breast cancer, intestinal cancer, liver cancer, and skin cancer.

Summarizing the studies worldwide, tea drinking has the following eight benefits: (a) refreshing and wakening, elimination of tiredness and increase in energy, (b) diuretic, (c) lowering of blood cholesterol and low-density lipoprotein levels, (d) prevention of caries in teeth, (e) strengthening of the walls of blood vessels, (f) antibacterial effect, (g) antimutagenesis and anticarcinogenesis, and (h) antiaging effect.

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Whiskey

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I. INTRODUCTION

Whiskey (1–5, 8, 12, 16, 19–22) (Gaelic: *uisge beatha* = water of life) is classified as a spirit made from cereal starch, the manufacture of which involves hydrolytic breakdown of the starch into fermentable sugars, followed by fermentation, distillation, and maturation. Whiskies themselves may be classified on the basis of the cereal used (wheat, barley, corn, rye), on the degree of blending, and on the country of origin: almost all whiskey is produced in Scotland, Ireland, Canada, and the United States. Malt (Scotch) whiskey (from barley only) is manufactured in the Scottish highlands and west coast. Grain whiskey is manufactured (for instance, in the Scottish lowlands) essentially from cereals other than barley. In Ireland the manufacture of whiskey is from mixtures of malted and unmalted barley, together with other cereals such as corn, rye, and wheat. Rye whiskey is made in Canada, using rye as the principal cereal, and in the United States, corn (at least 51% of the total cereal grain used) is the principal cereal for the manufacture of Bourbon whiskey. By definition, all whiskey production must include mashing to produce wort, fermentation, distillation, and maturation (Fig. 1 and Table 1). Every whiskey distillery is unique in its own right, yet the respective processing procedures actually have more similarities to each other than differences. As examples of specific differences, the production of specific whiskies may include, for instance, mixtures of cereals for the fermenting mash, three distillation steps rather than two (Irish whiskey), and charcoal-mellowing the distillate before maturation (Tennessee whiskey). In contrast, there are other variables in whiskey production, especially in relation to the blending of whiskies, that still remain confidential to a particular manufacturer. In the raw cereals, the essential component for whiskey production is the starch content, which is approximately 70% on a dry weight basis.

II. MALTING AND KILNING (MALT WHISKY)

The barley is cleaned and then steeped in cold water for 2 to 3 days. After soaking, the water is drained off and the grain is spread out to a depth of 2 to 3 feet on the malting floor, providing conditions for the promotion of germination, namely 24°C over a period

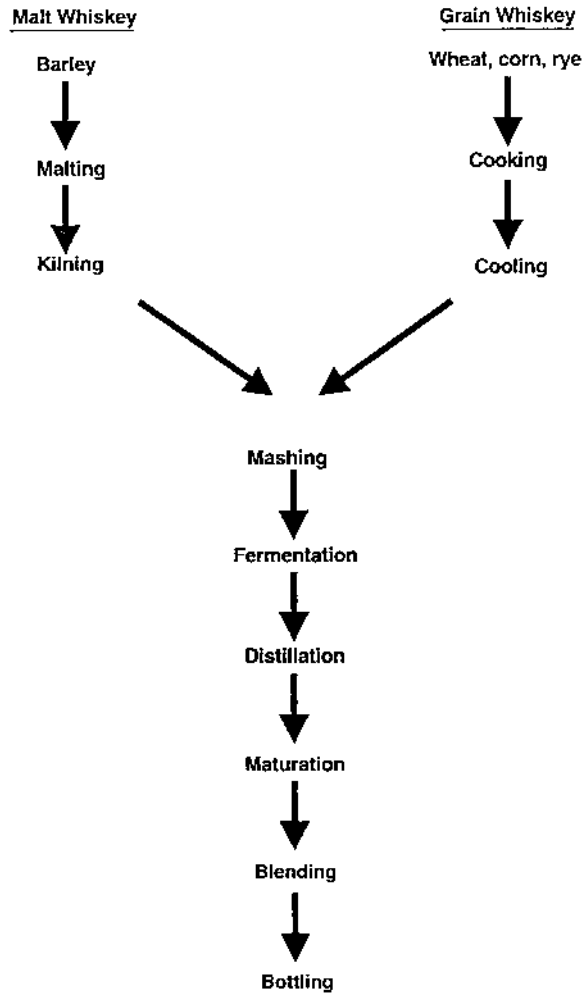

Figure 1 Flow chart for whiskey manufacture.

Table 1 Processing Steps and Application Principles for Whiskey Processing

Stages of processing	Application principles
Raw material	Starch content
Pretreatment before mashing	Amylases; β-glucanase; proteases; starch gelatinization
Mashing	Hydrolysis of starch; solubilization of sugars and amino acids
Fermentation	Ethanol formation
Distillation	Recovery of ethanol
Maturation	Flavor development
Blending	Mixtures of malt and grain whiskies
Bottling	Dilution of matured spirit

of 8 to 12 days. Temperature control is critical and the temperature is maintained either by constant manual turning of the spread grain or by large drums that have cool air blown through them. This germination procedure results in an increase in the levels of the activities of the α-amylase and β-amylase enzymes. These enzymes catalyze the hydrolytic breakdown of starch and are specific towards the α-1,4-glucosidic linkages of the amylose and amylopectin components of starch. They differ in their modes of action: α-amylase carries out internal random hydrolysis, whereas β-amylase removes two glucose residues at a time from the nonreducing end of the polysaccharide chain. The principal end-product of the joint action of α- and β-amylases upon starch is the fermentable sugar maltose. Some dextrin is also produced because neither of these amylases hydrolyzes the α-1,6-glucosidic linkage branch points of amylopectin.

Malting also encourages the development of β-glucanase and protease activities. These enzymes act on components of the starchy endosperm (84% of the total barley grain), respectively the endosperm cell wall structure and the storage proteins (12% of the raw cereal on a dry-weight basis), to enable germination to take place.

The sprouted grain is subjected to kilning, in which the grain is heated gradually to approximately 52°C in order to stop germination but not to destroy the enzymes. In the manufacture of Scotch, the germinated barley (malt) is dried in the presence of smoke rising from a peat fire situated 10 to 15 feet below the malt. The smoke contributes a special peaty flavor (in chemical terms, phenolic compounds, specifically phenol, isomeric cresols, xylenols, and guaiacol) to the malt. At the end of the kilning step, the moisture level of the malt has declined to approximately 5%. The conditions of malt drying favor the Maillard reaction, which contributes to browning and to flavor development. The dry malt may be stored until required.

III. MASHING

The dried malt is cleaned and then milled to form the grist. The grist is mixed with water at approximately 68°C in mash tuns for 0.5 to 1.5 hr. During mashing, the milled malt and water are subjected to continuous agitation; and, in addition, four batches of water, each hotter than the preceding batch, are used to attain maximum extraction of soluble compounds from the malt. The process of mashing is characterized chemically by the continuing hydrolysis of starch by amylase activity into fermentable sugars, chiefly maltose, with smaller amounts of sucrose, glucose, and fructose. The other principal purpose of mashing is to bring into solution not only the sugars but also amino acids, resulting from protease activity, which are to be a nitrogen source for yeast growth during fermentation.

At the end of the mashing period, the resulting liquor (wort) is recovered by filtration, while the remaining residue (draff) is used as cattle feed.

IV. GRAIN WHISKEY

In the production of grain whiskey, an initial cooking stage at 120°C for 1.5 hr is required in order to gelatinize the starch of cereals other than barley. The resulting mash is cooled to 60–65°C before the addition of 10–15% of freshly malted barley, which provides the amylase activity for the hydrolysis of the gelatinized starch.

V. FERMENTATION

The wort is cooled to 20–25°C and then inoculated with yeast (*Saccharomyces cerevisiae*). Fermentation, in wooden vessels of diameter 4 m and height of 5 m, and a capacity of 7500 to 45,000 liters, requires 2 to 3 days and is principally characterized by the growth of the yeast and the fermentation (through the Embden-Meyerhof-Parnas, or glycolytic, metabolic pathway) of the wort sugars to ethanol (Fig. 2).

The pH of the fermenting medium may decrease from 5.0–5.5 to 4.2–4.5 due to the production of organic acids, especially acetic, succinic, and pyruvic acids. Many other compounds, such as higher alcohols, carboxylic acids, esters, aldehydes, and ketones, are formed at this time: these compounds are sufficiently volatile to carry over into the distillate and therefore to make a major contribution to the final flavor of the whiskey. Glycerol, also formed during fermentation, is nonvolatile (b.p. 290°C) and therefore is nondistillable organic material that might otherwise have contributed to whiskey flavor. The yeast, in addition, has the ability to convert sulfur-containing amino acids into volatile sulfur-containing flavor compounds such as dimethyl disulfide (CH_3-S-S-CH_3).

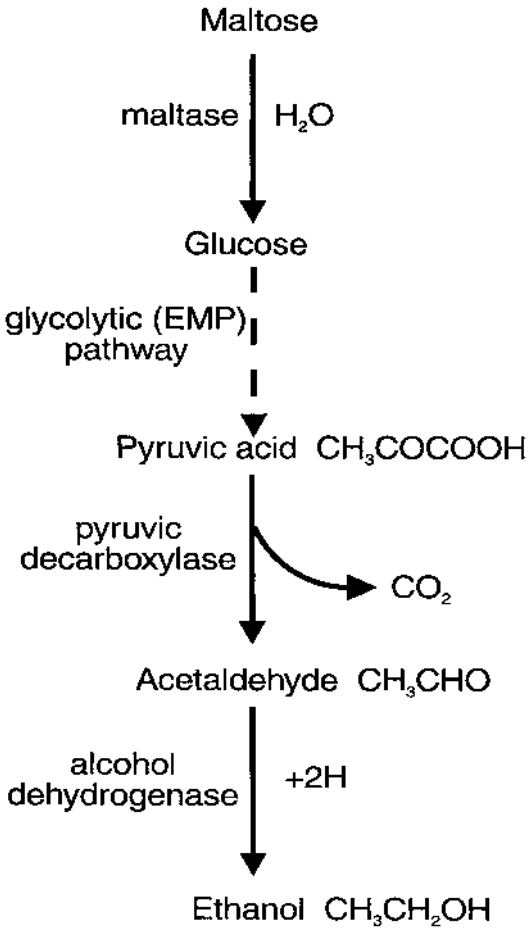

Figure 2 Fermentation of maltose to ethanol and carbon dioxide.

In whiskey, an important component in terms of contribution to flavor is a small optimal amount of the fusel oil fraction, which consists of n-amyl alcohol, isoamyl alcohol, butanol, isobutanol, and propanol. The principal components of the fusel oil fraction are the two amyl alcohols that originate during fermentation as a result of the deamination of leucine and isoleucine. These amino acids in turn arise from the hydrolysis of grain proteins and of autolyzed yeast protein.

The product of this fermentation is a liquid (the wash) that contains about 10% ethanol. The carbon dioxide produced during fermentation is recovered and used, for instance, in the manufacture of soft drinks.

VI. DISTILLATION

A. Malt Whisky

The production of malt whisky involves batch-distillation of the fermented liquid in two copper pot stills, namely the wash still and the spirit still (Fig. 3).

The contents of the fermentation vat are transferred to a large wash still, of capacity 15,000 to 30,000 liters, and boiled for 5–6 hr to produce a distillate (the low wines) that contains 20–25% ethanol (35–45° proof British). The low wines are distilled in the spirit still to produce the following three fractions:

1. Foreshots
2. Whisky (potable spirit containing 63–71% ethanol: 120–150° proof British)
3. Feints (approximately 25% ethanol).

The distillate is run through a spirit safe, prior to maturation, in order to determine the quality of the spirit. The ethanol content is determined by hydrometry because ethanol possesses a specific gravity lower than that of water.

The careful selection of the cut points during this distillation procedure greatly influences the flavor of the final product. The first cut from foreshots to spirit governs the level of aldehydes and short-chain esters; the second cut (spirit to feints) determines the concentrations of higher alcohols and acids. The foreshots and feints are returned to

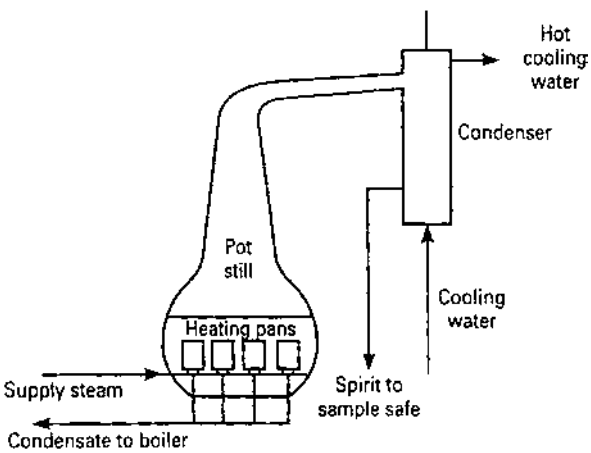

Figure 3 Pot still for batch distillation in the production of malt whiskey. (From Ref. 5.)

the process, being redistilled in the spirit still with the next batch of low wines. The conditions of distillation also enable further reactions to occur with respect to whisky flavor development:

1. Continuing ester formation
2. Maillard reaction and the accompanying Strecker degradation to produce aldehydes (especially acetaldehyde, 2-furaldehyde, 5-hydroxymethyl-2-furaldehyde)
3. Continuing formation of volatile sulfur-containing compounds from corresponding nonvolatile precursors
4. The conversion of β-hydroxypropionaldehyde to acrolein:

$$HOCH_2CH_2CHO \xrightarrow[-H_2O]{} CH_2{=}CHCHO$$

5. The oxidation of unsaturated fatty acids (derived from barley malt) to carbonyl compounds.

B. Grain Whiskey

Grain whiskey is normally produced by continuous distillation in a Coffey (patent) still, which consists of an analyzer column and a rectifier column and which in fact has not greatly changed since it was first developed by Aeneas Coffey in 1831 (Fig. 4).

In this distillation system, the incoming material is the cold wash of 10% ethanol content, and the products from this process are as follows:

1. A low-boiling-point fraction (the heads) rich in aldehydes and esters, from which any ethanol present can be recovered.
2. Raw spirit (main fraction) containing ethanol, approximately 95% by volume (190° proof U.S.). In practice, the whiskey is actually distilled at 180–186° U.S. proof because United States law does not permit distillation at over 190° U.S. proof (166° proof British).
3. A higher-boiling fraction that is rich in fusel oil components (Table 2)

The distillation of aqueous ethanolic mixtures is governed by specific physical properties of water and ethanol, most notably that an aqueous ethanolic mixture cannot be completely separated into ethanol and water. These are liquids that are completely miscible with each other and, therefore, out of all the possible ethanol-water mixture compositions, there is one particular mixture (96% ethanol and 4% water) that has a maximum vapor pressure and that therefore boils at a lower temperature than any other mixture of these two liquids. This mixture is an azeotropic (constant boiling) mixture that is able to distil without changing its composition; that is, the distillate has the same composition as the original liquid. In this case it is a minimum boiling mixture (a particular kind of azeotropic mixture) because the boiling point is lower than for any other mixture of ethanol and water (15).

In the Coffey still, the wash is steam-distilled and the resulting vapor is richer in ethanol than the original wash. However, in the rectifier column, the principal distillate fraction recovered is the azeotropic ethanol-water mixture (raw spirit) containing approximately 95% ethanol.

After distillation, the spirit is diluted with distilled water: the ethanol content (percent by volume) of malt whisky is reduced to 63.5% (20), while that of grain whiskey is reduced to 55% before being placed into oak barrels for maturation.

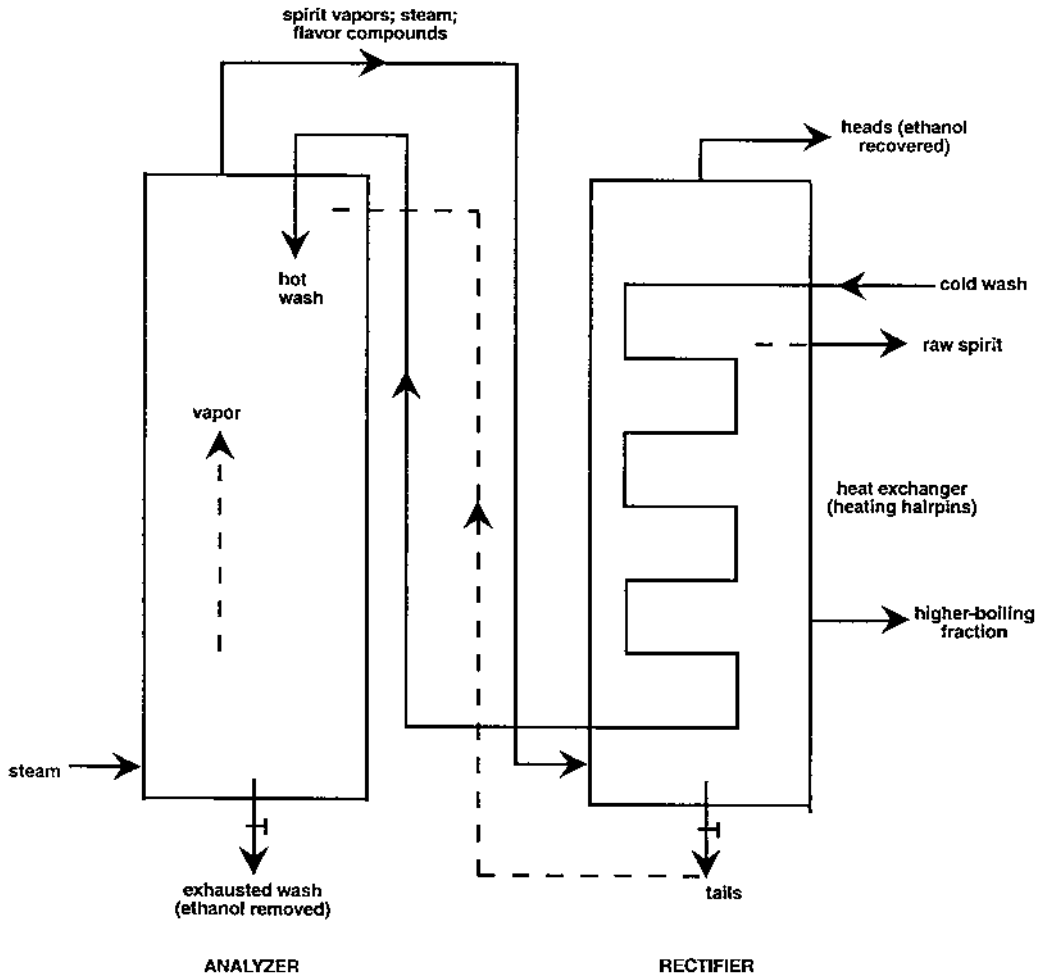

Figure 4 Coffey still for continuous distillation in the production of grain whiskey. (From Ref. 5.)

Table 2 Aliphatic Alcohols in Relation to Their Recovery from Continuous Distillation in the Coffey Still

Alcohol	Boiling point (°C)	Fraction
Methanol	64.7	Light (heads)
Ethanol	78.3	Raw spirit
n-Propanol	97.2	Higher boiling
n-Butanol	117.7	Higher boiling
Isobutanol	107.9	Higher boiling
n-Amyl alcohol	138.0	Higher boiling
Isoamyl alcohol	131.5	Higher boiling

VII. MATURATION (17)

The whiskey obtained directly from the distillation step is colorless and has a harsh taste, and therefore a long period of maturation is essential to develop the final flavor, which is a major determinant of whiskey quality. The diluted raw spirit is stored in air-permeable oak barrels (casks) in temperature-controlled government-bonded warehouses for up to 12 or 15 years, sometimes even longer: a minimum maturation period of 3 years is mandatory for Scotch and Canadian whiskies. There are five sizes of oak cask that are used for bulk whiskey (Table 3).

It is this maturation step that distinguishes whiskey from rawer spirits such as gin and vodka. Maturation, the progress of which is governed by the factors of temperature, time, and humidity, is characterized by gradual changes in the flavor and color of the maturing spirit, together with a concurrent decline in both volume and alcoholic content. There is also at this time a corresponding decline in the level of volatile sulfur-containing flavor compounds that were generated during the fermentation and distillation stages. The flavor changes occur essentially as a result of reaction among the chemical components both of the barrel wood and of the raw distillate, and continuing interaction between these two sets of chemical components.

Barrels used in whiskey maturation are made either from American white oak (*Quercus albus*) or from oaks of European origin (*Q. petraea*; *Q. robur*). Not only is the original wood important, but a key step in barrel manufacture is the heat-charring (over 200°C) of the inside of the barrel, a procedure that brings about the degradation of the major structural components of the wood. In addition, the whiskey industry uses recharred (regenerated) barrels that had previously been used for the maturation of other alcoholic beverages, especially sherry.

In chemical terms, the maturation of whiskey is far from being fully understood. However, the degradation of the structural components of oak heartwood by heat-charring may be outlined as follows:

1. Lignin to methoxy (for example, vanillin;see Fig. 5) and dimethoxy phenols, and cresols
2. Hemicellulose to hexoses and pentoses, which lose molecules of water to become furfural and hydroxymethyl furfural
3. Cellulose to D-glucose
4. Maillard reaction with its accompanying Strecker degradation: eventual production of pyrazines, pyrroles (Fig. 5), and pyridines, together with the direct heat pyrolysis of sugars

Table 3 Oak Casks Used in the Maturation of Bulk Whiskey

Cask	Approximate capacity (liters)	Remarks
Butt	364 or more	Sherry casks for malt whisky
Hogshead	205–364	Sherry casks for malt whisky
Barrel	160–205	For American grain whiskey
Quarter	127–159	
Octave	41–68	

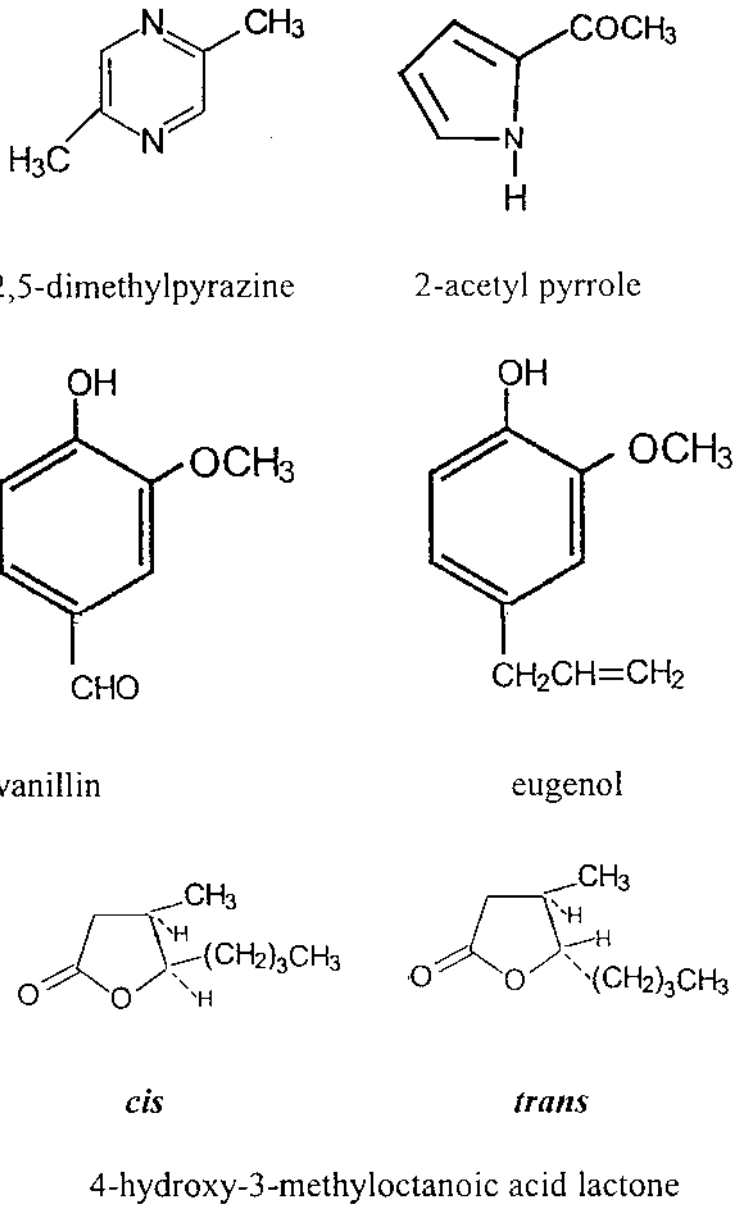

Figure 5 The structures of several compounds that impart unique flavors to whiskey during maturation.

In addition to the structural compounds, oak heartwood contains extractives, especially phenolic compounds, fatty acids, and lactones, which become extracted directly into the raw spirit during maturation.

All of these low-molecular-weight compounds are highly soluble in aqueous ethanol and such extraction, into the spirit, of these compounds originating from the wood of the barrel is an important part of maturation.

The species of oak used in barrel construction also influences the final whiskey flavor. The use of wood from *Q. alba* results in increased levels of vanillin and of lactones, especially the *cis* and *trans* isomers of 4-hydroxy-3-methyloctanoic acid lactone (also known as 3-methyl-4-hydroxy caprylic acid γ-lactone, β-methyl-γ-octalactone, whiskey lactone, or oak lactone;see Fig. 5), in the maturing whiskey, where they impart a woody aroma, whereas the concentration of tannins is increased with the use of wood from European oaks. In this context, a characteristic component of oak-matured spirits is eugenol which imparts a clove-like flavor (see Fig. 5) (11,13,14,18).

The use of old sherry casks for whiskey maturation increases in its own right the level of tannins and of sugars such as glucose, arabinose, and xylose in the maturing spirit.

During maturation, the very many chemical reactions, involving both the components of the distillate and the wood-derived compounds, may be variously described as oxido-reductions, esterifications, Maillard reactions, polymerizations, and polycondensations. The first group of reactions are the most important, especially since the oak barrels are permeable to oxygen, and may be exemplified by the oxidation of some of the ethanol to acetaldehyde and acetic acid with the subsequent formation of ethyl acetate.

Maturation over a period of several years results also in the development of the amber color, which for the purpose of consumer acceptability may be standardized with added caramel.

VIII. BLENDING

Malt whisky by itself has a heavy flavor and is therefore frequently blended with grain whiskey to increase its general acceptability. The most frequent composition of blended whiskey is 60–70% grain whiskey and 30–40% malt whisky.

IX. WHISKEY FLAVOR COMPONENTS

The ethanol content excepted, the most important property, and hence quality criterion, of whiskey is the flavor, which is governed by the presence and relative concentrations of a large number of organic compounds (congeners), which together are less than 1% of the total whiskey composition (6). The detailed congeneric content of three blended types of whiskey is presented in Table 4.

In whiskey, carboxylic acids are present: acetic acid accounts for 50–90% of the total volatile acid fraction. Other acids present are short-chain saturated volatile fatty acids (octanoic (caprylic) acid 8:0, decanoic (capric) acid 10:0, dodecanoic (lauric) acid 12:0), and lactic and succinic acids.

The conditions of fermentation, distillation, and maturation favor the interaction of alcohols and organic acids to produce esters, which are volatile and possess distinct aromas. The ethyl esters of the preceding acids are particularly well represented and the most important ester is ethyl acetate (b.p. 77.1°C), formed by the interaction of ethanol and acetic acid.

The predominant aldehyde in whiskey is acetaldehyde, but other aldehydes are present, especially furfural (2-furaldehyde) and hydroxymethyl furfural (HMF; 5-hydroxymethyl-2-furaldehyde).

Acetals such as 1,1-diethoxyethane (diethyl acetal) also occur in whiskey; they arise, during distillation and maturation, from the interaction of acetaldehyde and ethanol in acid conditions.

Table 4 Congeneric Content of Major Types of Distilled Alcoholic Beverages[a]

Component[b]	American blended whiskey	Canadian blended whiskey	Scotch blended whiskey
Fusel oil	83	58	143
Total acids (as acetic acid)	30	20	15
Esters (as ethyl acetate)	17	14	17
Aldehydes (as acetaldehyde)	2.7	2.9	4.5
Furfural	0.33	0.11	0.11
Total solids	112	97	127
Tannins	21	18	8
Total congeners (%w/v)	0.116	0.085	0.160

[a]Above determinations made by AOAC official methods.
[b]Grams per 100 liters at 100° proof.
Source: Ref. 20. Reproduced with permission. Copyright © by Carl W. Hall.

Flavor profiles of whiskey are determined by gas chromatography (GC) for the volatile components (6,9) and by high-performance liquid chromatography for the relatively nonvolatile components. Because each flavor component occurs at the nanogram level or lower, final identification may have to be confirmed by mass spectrometry or by infrared spectroscopy. GC analysis has been able to identify 313 different volatile compounds, including 32 alcohols and 22 esters, from Scotch whiskey (7), and such objective information may complement the more traditional but subjective organoleptic testing. The number of flavor components tends to be fewer in grain whiskey than in malt whiskey (10).

The conditions of whiskey manufacture greatly encourage the formation of many compounds from the materials used in production, and the subsequent interaction of these compounds. Increasing knowledge of the chemistry of whiskey manufacture may result in closer correlation, in terms of quality control, between objective chemical analysis, for example GC profiles, and subjective sensory evaluation.

X. BOTTLING

The matured spirit is further diluted with water to 40–43% by volume of ethanol (80–86° U.S. proof) before being bottled. Therefore, in terms of quantity, the major component of whiskey is actually water (approximately 60% by volume). Once bottled, the whiskey does not undergo subsequent change because oxygen no longer has access to the matured spirit. This represents the termination of maturation, but on the other hand there is no decline in whiskey quality due to age.

ACKNOWLEDGMENTS

The information in this chapter has been modified from Food Chemistry Workbook II, edited by J. S. Smith, ®2000, Science Technology System, West Sacramento, California. Used with permission.

APPENDIX: PROOF STRENGTH OF DISTILLED SPIRITS

British: 100% ethanol by volume ≡ 175° proof.
100° proof British ≡ 57.1% ethanol by volume.
U.S. Ethanol content (% by volume) of a liquid at 60°F (15.56°C) multiplied by 2. For example, 95% ethanol by volume ≡ 190° U.S. proof.

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