Innovations in Agricultural & Biological Engineering

Dairy Engineering

Advanced Technologies and Their Applications

Editors Murlidhar Meghwal
Megh R. Goyal
Rupesh S. Chavan

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DAIRY ENGINEERING

Advanced Technologies and Their Applications

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DAIRY ENGINEERING

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Edited by

Murlidhar Meghwal, PhD

Megh R. Goyal, PhD, PE

Rupesh S. Chavan, PhD

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LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
ACF	aberrant crypt foci
ACMSF	Advisory Committee on the Microbiological Safety of Food
ADI	acceptable daily intake
AP	aspartic proteases
APP	aseptic processing and packaging
AQIS	Australian Quarantine and Inspection Service
ASSOCHAM	Associated Chambers of Commerce and Industry
ATP	adenosine triphosphate
BIS	Bureau of Indian Standards
CAGR	compound annual growth rate
CAP	Common Market Organizations
CBM	*Clostridium butyricum* MIYAIRI
CBM	continuous *basundi* making machine
CCK	cholecystokinin
CFDA	China Food and Drug Administration
CFIA	Canadian Food Inspection Agency
CFSAN	Center for Food Safety and Applied Nutrition
CG	Central Government
CII	Confederation of Indian Industry
CLA	conjugated linoleic acid
CP	cysteine proteases
CPV	conical process vat
CRZ	coastal regulation zone
CVD	cardiovascular disease
DIPP	Department of Industrial Policy and Promotion
DPP	dipeptidyl peptidase
EC	essential commodities
EEA	European Economic Area Agreement
EFSA	European Food Safety Authority
EP	European Patent
ERED	electromagnetic radiation emitting devices
ES	emulsifying salts

FBO	Food Business Operators
FCI	Food Corporation of India
FCS	food category system
FDA	Food and Drug Administrator
FFA	free fatty acid
FICCI	Federation of Indian Chambers of Commerce and Industry
FIFO	first in, first out
FPC	fermentation-produced chymosin
FPO	Fruit Product Order
FSANZ	Food Standards Australia New Zealand
FSSA	Food Safety and Standard Act
FSSAI	Food Safety and Standards Authority of India
FSSR	Food Safety and Standards Regulations
GM	good manufacturing practices
GMP	glycomacropeptide
GMPs	good manufacturing practices
GOI	Government of India
GRAS	generally recognized as safe
HCl	hydrochloric acid
HMF	hydroxymethyl furfural
HPP	high hydrostatic pressure processing
HTST	high temperature for short time
IICPT	Indian Institute of Crop Processing and Technology
IIPH	Indian Institute of Public Health
ISI	Indian Standards Institution
LAB	lactic acid bacteria
LBG	Locust bean gum
LMP	low methoxyl pectin
LTLT	low temperature for longer time
MCA	milk-clotting activity
MCA	mozzarella cheese analog
MCI	milk clotting index
MFGM	milk lipoprotein lipase is protected in milk fat globular membrane
MMPO	Milk and Milk Products Order
MPI	Ministry for Primary Industries
MSME	Ministry of Micro, Small & Medium Enterprises
MUL	maximum use limits
NISIET	National Institute of Small Industry Extension Training
NSSO	National Sample Survey Organisation

NZFSA	New Zealand Food Safety Authority
NIFTEM	National Institute of Food Technology Entrepreneurship and Management
PA	proteolytic activity
PCT	patent cooperation treaty
PDO	protected designation of origin
PEF	pulse electric field
PFA	Prevention of Food Adulteration Act
PFB	petty food business
PG	poly-galacturonase
PMRY	Prime Minister's Rozgar Yojana
PMSF	phenyl methyl sulfonyl fluoride
POL	polyphosphate
QCI	Quality Council of India
REGP	Rural Employment Generation Program
RO	reverse osmosis
RTD	residence time distribution
RTE	ready-to-eat segment
SBP	systolic blood pressure
SEM	scanning electron microscope
SFDA	State Food and Drug Administration
SG	State Governments
SNF	solids not fat
SOP	standard operating procedure
SP	serine proteases
SSHE	scraped-surface heat exchanger
TBI	Technology Business Incubation
TIDPS	traditional Indian dairy products
TMA-N	trimethylamine nitrogen
TNF	tumor necrosis factor
TSC	tri-sodium citrate
TSPP	tetra-Na-pyrophosphate
TTHEs	triple tube heat exchangers
UF	ultrafiltration
UHT	ultra-high temperature
USFDA	United States Food and Drug Administration

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PREFACE 1

The dairy industry includes various activities, such as farming for fodder for cattle; cattle rising and care; milk production; transportation; packaging; and distribution. According to the Ministry of Agriculture—Government of India, there are more than 140 million tons of milk production in the year 2014–2015; and India is the largest milk-producing country in the world. The major milk-based products consumed all over the globe are aarts, amasi, ayran, baked-milk, basundi, bhuna-khoya, blaand, booza, yogurt, butter, butterfat, buttermilk, buttermilk-koldskal, buttermilk-powder, cacik, camel-milk, casein, caudle, chaas, chal, chalap, cheese, clabber, clotted-cream, condensed-milk, cottage-cheese, cream, cream-cheese, crème-anglaise, cuajada, curd, curd-snack, custard, dadiah, daigo, dondurma, fermented milk products, frozen custard, frozen yogurt, infant formula, kefir, khoa, kulfi, lassi, malai, mithadahi, paneer, and whey.

According to latest statistics of the International Dairy Federation 2015, about one billion people in the world live on dairy farms; milk production was estimated at 748.7 million tons in 2011; the gross production value of raw milk produced across the world was 292 billion US$; the trade of milk products equals around 64 billion US$ in terms of value, globally. Around 150 million small-scale dairy households, equivalent to 750 million people, are engaged in milk production, and consumption of dairy products is expected to increase by 20% or more before 2021. It reveals huge potential of this sector.

This book volume provides the technology, suggests devices, standardization, packaging, ingredients, laws and regulatory guidelines, and information on infrastructure to transform raw milk into highly value-added products.

In the food processing industry, dairy industry is the one of the major partners. Dairy industry will be able to use emerging and innovative technologies given in this book to meet the changing demands of a growing and increasingly diverse population. As convenience and health consciousness become more important, consumers are increasingly demanding highlyprocessed and value-added dairy-based foods products such as emulsified salted cheese products, cheese made from plant-based coagulants, value-added khoa, high-pressure processed dairy products, and probiotics. This kind of

change in consumption patterns will lead to the development of more innovative and processed foods (especially dairy-based food items), and it will not only affect the domestic market but also will influence the international trade. The increasing size, transportation, internet facility, media, and diversity of the global population have driven demand for a greater variety of dairy-based foods products. The combination of expanding export markets and shifting and increasing domestic consumption will lead to significant changes throughout the dairy manufacturing industry. This book is written for dairy and food engineers. Most of the contributors are by profession engaged in dairy and food processing related production, research, training, and teaching.

The targeted audience for the book is practicing engineers, researchers, lecturers, teachers, professors, dairy and food professionals, students of these fields, and all those who have inclination for the dairy and food processing sector. Hopefully, it covers all people because everybody consumes dairy-based products and food. Because the book not only covers the practical aspect, but also has a lot of basic information and is instructive, students in undergraduate, graduate, and post-graduate courses and post-doctoral researchers will also benefit.

Part I on "*Advanced Applications in Dairy Engineering*" presents chapters on plant-based coagulants in cheese making: review; rheology of fruit ripple; and role of emulsifying salts in cheese products. Part II is focused on "*Process Engineering—Dairy Products*," which has chapters on dairy engineering—milk processing and milk products; aseptic food processing and packaging; high-pressure processing of dairy products; and introspection on mechanization of traditional Indian dairy products. Part III covers "*Human Health Benefits from Various Dairy Products*" such as the health benefits of milk-derived bioactive peptides and potential human health benefits of probiotics. Part IV on "*Food Laws, Acts, Orders and Regulations*" covers food laws and their implications and also covers food laws all over the world. This section also provides the details about entrepreneurship and management of food plant.

In order for the book to be useful to engineers, coverage of each topic is comprehensive enough to serve as an overview of the most recent and relevant research and technology. Numerous references are included at the end of all chapters.

My own training and work experience as a dairy and food process engineer and teacher was crucial in conceiving this book on dairy engineering. Also, it was the contributors who did the real great work. Thanks to all

contributors for their time and energy to create these scholarly and practical chapters. Their professionalism is appreciated, and they have my utmost appreciation and admiration. My thanks also to Almighty God, whose love and blessings helped me immensely.

—Murlidhar Meghwal, PhD
December 2016

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PREFACE 2

<https://en.wikipedia.org/wiki/Dairy_farming> indicates that *dairy farming is a class of agriculture for long-term production of milk, which is processed (either on the farm or at a dairy plant, either of which may be called a dairy) for eventual sale of a dairy product. There is a great deal of variation in the pattern of dairy production worldwide. Many countries which are large producers consume most of this internally, while others (in particular New Zealand) export a large percentage of their production. Internal consumption is often in the form of liquid milk, while the bulk of international trade is in processed dairy products such as milk powder. Most milk-consuming countries have a local dairy farming industry, and most producing countries maintain significant tariffs to protect domestic producers from foreign competition but, the largest dairy exporting country, does not apply any subsidies to dairy production. The milking of cows was traditionally a labor-intensive operation and still is in less developed countries. Small farms need several people to milk and care for only a few dozen cows, though for many farms, these employees have traditionally been the children of the farm family, giving rise to the term "family farm." Advances in technology have mostly led to the radical redefinition of "family farms" in industrialized countries such as Australia, New Zealand, and the United States.*

With farms of hundreds of cows producing large volumes of milk, the larger and more efficient dairy farms are more able to weather severe changes in milk price and operate profitably, while "traditional" very small farms generally do not have the equity or cash flow to do so. The common public perception of large corporate farms supplanting smaller ones is generally a misconception, as many small family farms expand to take advantage of economies of scale and incorporate the business to limit the legal liabilities of the owners and simplify such things as tax management.

Worldwide, the largest milk producer is the European Union with its present 27 member countries, with more than 153,000,000 metric tons (151,000,000 long tons; 169,000,000 short tons) in 2009 (more than 95% cow milk). By country, the largest producer is India (more than 55% buffalo milk), the largest cow milk exporter is New Zealand, and the largest importer is China.

In the United States, the top five dairy states are, in order by total milk production, California, Wisconsin, New York, Idaho, and Pennsylvania.

Dairy farming is also an important industry in Florida, Minnesota, Ohio, and Vermont. There are 65,000 dairy farms in the United States. Pennsylvania has 8500 farms with 555,000 dairy cows. Milk produced in Pennsylvania yields annual revenue of about US$1.5 billion. Herd size in the United States varies between 1200 on the West Coast and Southwest, where large farms are commonplace, to roughly 50 in the Midwest and Northeast, where land-base is a significant limiting factor to herd size. The average herd size in the United States is about one hundred cows per farm but the median size is 900 cows with 49% of all cows residing on farms of 1000 or more cows.

A **dairy product** or *milk product* is food produced from the milk of mammals. Dairy products are usually high energy-yielding food products. A production plant for the processing of milk is called a dairy or a dairy factory. Apart from breastfed infants, the human consumption of dairy products is sourced primarily from the milk of cows, water buffaloes, goats, sheep, yaks, horses, camels, domestic buffaloes, and other mammals. Dairy products are commonly found throughout the world (see Appendix C at the end of this book volume).

I recall my childhood. I was breast fed by mother till I was 8 years old. It calmed me down from my nervousness and hypertension, and imparted me security in the lap of my mother. Till fifth grade, I never tasted the dairy

milk except few drops in an Indian tea. In sixth grade, once a day we were asked to sit on a 1 × 50 m mat and were served one 16 oz glass of milk by American Peace Corps volunteers. I enjoyed drinking not only the milk but also dedication of these volunteers. This milk was prepared from the powder milk that was imported from Europe. What a gesture to the undernourished students in the developing countries!

At the 49th annual meeting of the Indian Society of Agricultural Engineers at Punjab Agricultural University (PAU) during February 22–25 of 2015, a group of ABEs and FEs convinced me that there is a dire need to publish book volumes on focus areas of agricultural and biological engineering (ABE). This is how the idea was born on new book series titled "Innovations in Agricultural & Biological Engineering." This book on *Dairy Engineering: Advanced Technologies and Their Applications* is the fourth volume under this book series, and it contributes to the ocean of knowledge on dairy engineering.

The contributions by the cooperating authors to this book volume have been most valuable in the compilation. Their names are mentioned in each chapter and in the list of contributors. This book would not have been written without the valuable cooperation of these investigators; many of them are renowned scientists who have worked in the field of food engineering throughout their professional careers. I am glad to introduce Dr. Murlidhar Meghwal, who is an Assistant Professor in the Food Science and Technology Division, of the Center for Emerging Technologies at Jain University—Jain Global Campus in District Karnataka, India. With several awards and recognitions, including from President of India, Dr Meghwal brings his expertise and innovative ideas in this book series. Also, joining my team is Dr. Rupesh S. Chavan, who is a Senior Executive, Department of Quality Assurance, Mother Dairy Junagadh, Gujarat. He has also worked as Assistant Professor at the National Institute of Food Technology Entrepreneurship and Management, Kundli under the Ministry of Food Processing Industries, India; and In-charge of the International Bakery Research and Training Center. He is a professor/researcher and has specialized in dairy and bakery products. Without their support, leadership qualities as editors of the book volume and extraordinary work on dairy engineering applications, readers would not have this quality publication.

I will like to thank editorial staff, Sandy Jones Sickels, Vice President, and Ashish Kumar, Publisher and President at Apple Academic Press, Inc., for making every effort to publish the book. Special thanks are due to the AAP Production Staff for the quality production of this book.

I request that the reader offers his constructive suggestions that may help to improve the next edition.

I express my deep admiration to my family for their understanding and collaboration during the preparation of this book volume.

One of my college mates (Dr R. P. Singh) at PAU can be distinguished as among top five food engineers in USA. At present, Dr. Singh is a Distinguished Emeritus Professor of Food Engineering Department of Biological and Agricultural Engineering Department of Food Science and Technology University of California Davis, CA 95616, USA. I invite readers to consult him at rpsingh@ucdavis.edu, whenever they need. Can anyone live without food or milk?

As an educator, there is a piece of advice to one and all in the world: *"Permit that our almighty God, our Creator, provider of all and excellent Teacher, feed our life with Healthy Milk and Milk Products and His Grace—; and Get married to your profession—"*

—Megh R. Goyal, PhD, PE,
Senior Editor-in-Chief
December 2016

PREFACE 3

Food is defined as a "*substance, whether processed, partially processed or unprocessed, which is intended for human consumption.*" Dairy is an integral part of food industry and most of the diets are incomplete without dairy products. Dairy industry is changing rapidly and continuously, thus creating a demand of modern engineering with mechanization. The industry offers a lot of value-added products which include traditional as well as westernized products. Dairy industries thrive day in and day out to meet and fulfill the customer's needs, but due to changing age groups and eating habits, fulfilling the desires of the customers pose a huge challenge as well as an opportunity to the sector.

The intention of this book is to present knowledge on the dairy engineering, regulations, traditional dairy-based products and long-life products to the different segments of scholars, professors, researchers, and scientists in industries engaged in new product development. An attempt has been made to overcome the non-availability of a book in the field of dairy engineering focusing on the recent advances and emerging trends. This book volume, in short, explores and conveys the key concepts on dairy engineering and dairy products that are presented in four parts.

Part I: Advanced Applications in Dairy Engineering highlights the application of different processing aids including plant-based coagulants in cheese making and role of emulsifying salts in cheese-based products. Fruit ripples and the different factors governing the rheological parameters are also discussed in the subsection. Factors governing the consistency and quality of processed cheese (maturity of natural cheese, pH of cheese melt, type and concentration of emulsifying salts, processing temperature, speed of agitation, duration of heating, rate of cooling, temperature of storage, dry matter content, fat content, presence and concentration of ions, type and concentration of lactose or other sugars, and use of emulsifiers) are also discussed in detail along with the recent advancements in the commercial blends of emulsifying salts available in the market.

Part II: Process Engineering—Dairy Products highlights the application of recent and novel technologies for processing of dairy products including microfluidization, high pressure processing, and advancement in manufacturing of Indian traditional dairy products. The chapter on Dairy

Engineering encompasses the different milk processing techniques like thermal (pasteurization, sterilization) or non-thermal techniques (high pressure processing, pulse electric field), and distilled description of the equipments including chillers, centrifugal separator, homogenizer, microfluidizer, pasteurizer (batch and continuous), high hydrostatic processing machine, and pulse electric field setup. Aspects of aseptic processing and packaging are also presented in the subsection highlighting the different techniques for UHT treatment of milk and advancements in the aseptic packaging systems. HPP of milk has attracted attention of the consumers as it has least effect on flavor, color, and nutritional value, simultaneously, very effective in reducing the microbial growth and is presented in depth in the current subsection. Brief overviews on traditional and mechanized methods of Indian traditional products including khoa, khurchan, gulabjamun, peda, and others with their technological up-gradation, its shelf life, traditional sweets and storage is also presented in this subsection.

Part III: Human Health Benefits from Various Dairy Products includes the importance of bioactive peptides and application of unusual probiotics. In the present era of tailor-made diet, role of bioactive peptides which are derived from milk proteins are of great importance due to their adaptability by the human beings. Pharmaceutical and food industries are therefore showing a keen interest in manufacturing health foods which are either fortified with natural bioactive peptides or manufacturing of the food products like fermented food in which the bioactive peptides are released. In unusual probiotics chapter, apart from the most widely documented and universally used species of probiotics *viz. Lactobacillus* sp. and *Bifidobacterium* sp., there are other bacterial species with probiotic potential, which are presently being used or being tested for their safe utilization. Among them, *Bacillus* sp., *Weissella* sp., *Enterococcus* sp., *Clostridium* sp., *Butyrovibrio* sp., and yeasts are prominent which are briefly described in the chapter with their usage as unusual probiotics, their potential, and applications.

Part IV: Food Laws, Acts, Orders, and Regulations has its unique value and addresses the breakthrough about modern food laws and regulations. All over the world, there is a change in the perception about the methods which are suitable and appropriate for ensuring healthy foods for all consumers. Hence, the focus of Modern Food Laws is to lay down science-based standards, install comprehensive Food Monitoring Systems; ensure safe, hygienic, and wholesome food is produced by food manufacturers and distributed by food operators, and harmonization of standards and regulation of imported/exported foods. The section helps to understand

the Indian food laws and regulations in brief along with other regulations used worldwide.

It is my pleasure to express my heartful reverence to all the contributing authors for their versatile and scholastic approach toward the topics and constant co-operation throughout the progress of the book volume.

—Rupesh Chavan, PhD
December 2016

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FOREWORD 1

It is indeed a matter of great pleasure for me to know that this reference book on dairy engineering can spread a shining message of innovative and need based dairy engineering technologies for assisting in the three-dimensional development of students as entrepreneurs, academicians, and researchers.

The dairy industry is one of the constituent segments of the agro-based industry. Its frontier contribution to the National GDP depends on auto-mechanization and introduction of innovative technologies feasible for transformation of raw milk into new products exhibiting higher value addition in terms of nutrition and money. Dairying has become an important secondary source of income for millions of rural families in India. The accelerated socio-economic development during the 21st century is associated with challenging issues like food security, food safety, quality, and their linkages with national and international markets to compensate with increased demand of dairy/milk-based food products.

This book volume encompasses a good number of chapters on various aspects of dairy science and technology authored by scientists actively engaged in their respective area of specialization. The chapters in this book provide interesting insights to budding entrepreneurs, policymakers, investors, and students. It will become an excellent resource on the subject, especially in the Indian context.

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December 2016

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FOREWORD 2

I feel very delighted to write this foreword for this book, *Dairy Engineering: Advanced Technologies and Their Applications,* edited by Murlidhar Meghwal, Megh R. Goyal, and Rupesh S. Chavan, who are eminent scientists.

Dairy engineering is a discipline devoted to the collecting and processing of animal milk. In countries like India where small number of animals are being milked, the farmer may perform the functions of processing their own milk into salable dairy products, such as butter, cheese, or yogurt. As an attributive, the word dairy refers to milk-based products, derivatives and processes, and lots of engineering applications are involved to make the operations efficient.

Dairy engineering also involves storage, packaging, distribution and transportation of milk and dairy products along with principles of bacteriology, nutrition and biochemistry in order to develop the appropriate utilization of milk and their products. Processing includes managing of milk for supply or its alteration into dairy products. After the milk reaches the plant, processing work begins and it is converted into a variety of dairy products. The dairy industry plays a vital role in India's agro-based economy.

Dairy plants process the raw milk they receive from farmers so as to extend its marketable life. Two main types of processes are employed: heat treatment to ensure the safety of milk for human consumption and to lengthen its shelf-life, and dehydrating dairy products such as butter, hard cheese and milk powders so that they can be stored for longer time safely.

In this book, first three parts cover important topics on dairy science and engineering, such as: Plant-Based Coagulants for Cheese Making, Emulsifying Salts in Cheese Products, Milk Processing and Milk Products, Aseptic Processing and Packaging, High-Pressure Processing of Dairy Products, Mechanization of Production of Traditional Dairy Products and Their Standardization, Human Health Benefits of Dairy Products, Milk-Based Bioactive Peptides and Probiotics for Human Health. The Fourth Part covers Food Laws and Their Implications under Indian Conditions, Food Regulations around the Globe and also Entrepreneurship and Management of Dairy Products Processing Plant.

I congratulate the editors and Apple Academic Press Inc. for their timely decision of bringing out this book for use by scientists, engineers, professionals, and students. I am sure it will be a very useful reference book for professionals working in dairy sector.

R. T. Patil, PhD
Chief Technical Adviser—Khyati Foods Pvt. Ltd., Bhopal
Chairman & ED, Benevole Welfare Society for Post-harvest Technology, Bhopal
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Former Vice President, Indian Society of Agricultural Engineers, New Delhi

Bhopal, India
December 2016

WARNING/DISCLAIMER

Read Carefully

The goal of this book volume "*Dairy Engineering: Advanced Technologies and Their Applications*," is to guide the world community on how to manage efficiently for technology available for different processes in dairy engineering. The reader must be aware that the dedication, commitment, honesty, and sincerity are most important factors in a dynamic manner for a complete success. It is not a one-time reading of this compendium.

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He is a life member of the Indian Dairy Association (IDA), Society of Indian Bakers (SIB), and of SASNET-FF. He is engaged in education in dairy technology to the BTech (Food Technology and Management) and MTech students. He has attended subject-specific skill-oriented courses and training and several seminars, conferences and workshops. He is a Principal Investigator and Co-Principal Investigator for projects on milk and milk products funded by different esteemed agencies. He was a university topper during BTech (Dairy Technology). Dr Rupesh also holds a vast experience of research, manufacturing, and quality assurance in biscuit and milk industry. Readers may contact him at: rschavanb_tech@rediffmail.com

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ENDORSEMENTS FOR THIS BOOK VOLUME

Dairy Engineering: Advanced Technologies and Their Applications is a well-arranged and well-written book on recent advances in dairy engineering and science. This book will be very useful for dairy engineering professionals, industrialists, and undergraduate and postgraduate students.

—Tridib Kumar Goswami, PhD
Professor
Agricultural and Food Engineering Department,
Indian Institute of Technology, Kharagpur, WB, India

This book provides a comprehensive coverage of the various aspects of food engineering. Topics including modeling, food preservation, recent research on food engineering, and health-related aspects will be very useful to students and professionals in food engineering. Increasing awareness on food processing and preservation and the growing processed food market makes this book an excellent source for reference in these areas.

—Narendra Reddy, PhD
Professor and Ramalingaswami Fellow,
Centre for Emerging Technologies,
Jain University, Jain Global Campus,
Jakkasandra Post, Bangalore, India

This book has been written for a wide range of readership and will be very useful for dairy industry people to learn more about the products they make and food laws. Equally for academicians and researchers, this book will be a treasure trove. It also deals with products that have health benefits and are economical, as it will prevent wastage of milk.

—R. K. Sivanappan, PhD
Former Professor and Dean,
College of Agricultural Engineering & Technology,
Tamil Nadu Agricultural University (TNAU), Coimbatore, India

The topics in the book have been wisely selected; they cover not only the entire aspect of modern dairy engineering and processing, but also the health

benefits and various laws about dairy and food industries. This book would be useful to the students, researchers, and professionals in the field of dairy and food engineering.

—Soumitra Banerjee, PhD
Professor, Food Technology,
Centre for Emerging Technologies,
Jain University, Jakkasandra, Karnataka, India

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EDITORIAL

Apple Academic Press Inc. (AAP) will be publishing various book volumes on the focus areas under the book series titled *Innovations in Agricultural and Biological Engineering*. Over a span of 8–10 years, Apple Academic Press Inc. will publish subsequent volumes in the specialty areas defined by the *American Society of Agricultural and Biological Engineers* (<asabe.org>).

The mission of this series is to provide knowledge and techniques for agricultural and biological engineers (ABEs). The series aims to offer high-quality reference and academic content in **Agricultural and Biological Engineering** (ABE) that is accessible to academicians, researchers, scientists, university faculty, and university-level students, and professionals around the world. The following material has been edited/modified and reproduced below [From: "*Goyal, Megh R., 2006. Agricultural and biomedical engineering: Scope and opportunities. Paper Edu_47 Presentation at the Fourth LACCEI International Latin American and Caribbean Conference for Engineering and Technology (LACCEI' 2006): Breaking Frontiers and Barriers in Engineering: Education and Research by LACCEI University of Puerto Rico – Mayaguez Campus, Mayaguez, Puerto Rico, June 21–23*"]:

WHAT IS AGRICULTURAL AND BIOLOGICAL ENGINEERING (ABE)?

"*Agricultural Engineering (AE) involves application of engineering to production, processing, preservation and handling of food, fiber, and shelter. It also includes transfer of technology for the development and welfare of rural communities,*" according to <isae.in>. "*ABE is the discipline of engineering that applies engineering principles and the fundamental concepts of biology to agricultural and biological systems and tools, for the safe, efficient and environmentally sensitive production, processing, and management of agricultural, biological, food, and natural resources systems,*" according to <asabe.org>. "*AE is the branch of engineering involved with the design of farm machinery, with soil management, land development, and mechanization and automation of livestock farming, and with the efficient*

planting, harvesting, storage, and processing of farm commodities," definition by: <http://dictionary.reference.com/browse/agricultural+engineering>.

"AE incorporates many science disciplines and technology practices to the efficient production and processing of food, feed, fiber and fuels. It involves disciplines like mechanical engineering (agricultural machinery and automated machine systems), soil science (crop nutrient and fertilization, etc.), environmental sciences (drainage and irrigation), plant biology (seeding and plant growth management), animal science (farm animals and housing) etc.," by: <http://www.ABE.ncsu.edu/academic/agricultural-engineering.php>.

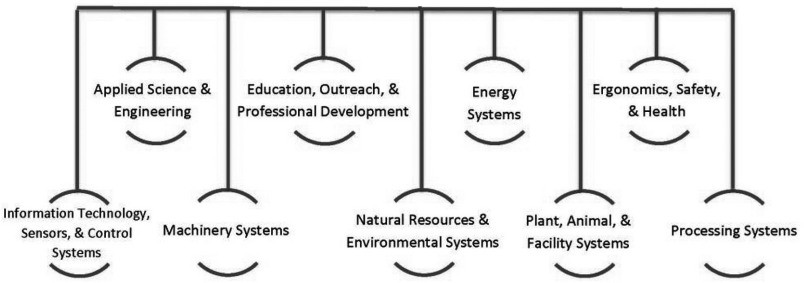
"According to https://en.wikipedia.org/wiki/Biological_engineering: "*BE* (*Biological Engineering) is a science-based discipline that applies concepts and methods of biology to solve real-world problems related to the life sciences or the application thereof. In this context, while traditional engineering applies physical and mathematical sciences to analyze, design and manufacture inanimate tools, structures and processes, biological engineering uses biology to study and advance applications of living systems.*"

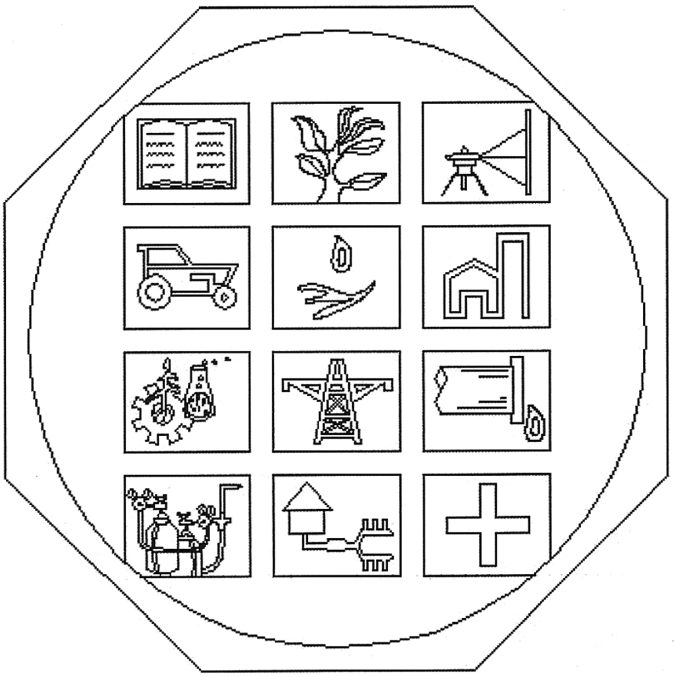
SPECIALTY AREAS OF ABE

Agricultural and Biological Engineers (ABEs) ensure that the world has the necessities of life including safe and plentiful food, clean air and water, renewable fuel and energy, safe working conditions, and a healthy environment by employing knowledge and expertise of sciences, both pure and applied, and engineering principles. Biological engineering applies engineering practices to problems and opportunities presented by living things and the natural environment in agriculture. BA engineers understand the interrelationships between technology and living systems, have available a wide variety of employment options. The <asabe.org> indicates that "*ABE embraces a variety of following specialty areas.*" As new technology and information emerge, specialty areas are created, and many overlap with one or more other areas.

1. **Aquacultural Engineering**: ABEs help design farm systems for raising fish and shellfish, as well as ornamental and bait fish. They specialize in water quality, biotechnology, machinery, natural resources, feeding and ventilation systems, and sanitation. They seek ways to reduce pollution from aquacultural discharges, to reduce

excess water use, and to improve farm systems. They also work with aquatic animal harvesting, sorting, and processing.





2. **Biological Engineering** applies engineering practices to problems and opportunities presented by living things and the natural environment.
3. **Energy:** ABEs identify and develop viable energy sources—biomass, methane, and vegetable oil, to name a few—and to make these and other systems cleaner and more efficient. These specialists

also develop energy conservation strategies to reduce costs and protect the environment, and they design traditional and alternative energy systems to meet the needs of agricultural operations.

4. **Farm Machinery and Power Engineering**: ABEs in this specialty focus on designing advanced equipment, making it more efficient and less demanding of our natural resources. They develop equipment for food processing, highly precise crop spraying, agricultural commodity and waste transport, and turf and landscape maintenance, as well as equipment for such specialized tasks as removing seaweed from beaches. This is in addition to the tractors, tillage equipment, irrigation equipment, and harvest equipment that have done so much to reduce the drudgery of farming.
5. **Food and Process Engineering:** Food and process engineers combine design expertise with manufacturing methods to develop economical and responsible processing solutions for industry. Also, food and process engineers look for ways to reduce waste by devising alternatives for treatment, disposal, and utilization.
6. **Forest Engineering**: ABEs apply engineering to solve natural resource and environment problems in forest production systems and related manufacturing industries. Engineering skills and expertise are needed to address problems related to equipment design and manufacturing, forest access systems design and construction; machine-soil interaction and erosion control; forest operations analysis and improvement; decision modeling; and wood product design and manufacturing.
7. **Information and Electrical Technologies engineering** is one of the most versatile areas of the ABE specialty areas, because it is applied to virtually all the others, from machinery design to soil testing to food quality and safety control. Geographic information systems, global positioning systems, machine instrumentation and controls, electromagnetics, bioinformatics, biorobotics, machine vision, sensors, and spectroscopy: These are some of the exciting information and electrical technologies being used today and being developed for the future.
8. **Natural Resources:** ABEs with environmental expertise work to better understand the complex mechanics of these resources, so that they can be used efficiently and without degradation. ABEs determine crop water requirements and design irrigation systems. They are experts in agricultural hydrology principles, such as controlling drainage, and they implement ways to control soil erosion and study

the environmental effects of sediment on stream quality. Natural resources engineers design, build, operate, and maintain water control structures for reservoirs, floodways and channels. They also work on water treatment systems, wetlands protection, and other water issues.

9. **Nursery and Greenhouse Engineering**: In many ways, nursery and greenhouse operations are microcosms of large-scale production agriculture, with many similar needs—irrigation, mechanization, disease and pest control, and nutrient application. However, other engineering needs also present themselves in nursery and greenhouse operations: equipment for transplantation; control systems for temperature, humidity, and ventilation; and plant biology issues, such as hydroponics, tissue culture, and seedling propagation methods. And, sometimes the challenges are extraterrestrial: ABEs at NASA are designing greenhouse systems to support a manned expedition to Mars!
10. **Safety and Health:** ABEs analyze health and injury data, the use and possible misuse of machines, and equipment compliance with standards and regulation. They constantly look for ways in which the safety of equipment, materials, and agricultural practices can be improved and for ways in which safety and health issues can be communicated to the public.
11. **Structures and Environment:** ABEs with expertise in structures and environment design animal housing, storage structures, and greenhouses, with ventilation systems, temperature and humidity controls, and structural strength appropriate for their climate and purpose. They also devise better practices and systems for storing, recovering, reusing, and transporting waste products.

CAREER IN AGRICULTURAL AND BIOLOGICAL ENGINEERING

One will find that university ABE programs have many names, such as biological systems engineering, bioresource engineering, environmental engineering, forest engineering, or food and process engineering. Whatever the title, the typical curriculum begins with courses in writing, social sciences, and economics, along with mathematics (calculus and statistics), chemistry, physics, and biology. Student gains a fundamental knowledge of the life sciences and how biological systems interact with their environment. One also takes engineering courses, such as thermodynamics, mechanics,

instrumentation and controls, electronics and electrical circuits, and engineering design. Then student adds courses related to particular interests, perhaps including mechanization, soil and water resource management, food and process engineering, industrial microbiology, biological engineering, or pest management. As seniors, engineering students work in a team to design, build, and test new processes or products.

For more information on this series, readers may contact:

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PART I
Advanced Applications in Dairy Engineering

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CHAPTER 1

PLANT-BASED COAGULANTS IN CHEESE MAKING: REVIEW

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CONTENTS

ABSTRACT

Cheese production essentially involves the use of milk-coagulating proteases. Destabilization of the casein micelles to cause gelation or coagulation of milk by targeting κ-casein is the main function of the enzymes in rennet. Understanding of fundamental mechanism behind enzymatic κ-casein cleavage has increased substantially. Difficulty in catering the escalating global cheese demand has expanded the search for suitable novel and economic milk coagulants for animal rennets. Fermentation-produced chymosin (FPC), microbial, and plant proteases are being used as rennet substitutes. Plant-derived enzymes have been extensively investigated as potential coagulants in cheese making. However, due to characteristic excessive proteolytic activity (PA) that lowers yield and acceptability of flavors in the final product, majority of them have been found to be unsuitable for cheese production. Technological advances have contributed to improve the performance of some of these enzymes in cheese making. In this chapter, plant-derived milk coagulants, their sources, and characteristics are summarized. Even with accumulated information on plant coagulants, in reality their application in cheese making is still in infancy.

1.1 INTRODUCTION

Cheese production is one of the earliest biotechnological enzyme applications.[141] The world cheese production has increased from 8.7 million MT in 1980 to 20 million MT mark in 2011 and this growing trend has continued in 2012 and 2013.[116] Milk coagulation is the main step for producing cheese. Preparations of proteolytic enzymes have been used to initiate this first step that is, the clotting of milk. Historically, most of these enzyme preparations have been extracts from the stomachs of ruminant, especially calf stomachs, which mainly comprise of chymosin and pepsin as clotting agents. The name "rennet" (animal rennin) is reserved for the enzyme preparations from ruminant stomachs, whereas other milk-clotting enzymes are generally named as "coagulants" with a few exceptions.[58] Escalating global demand for cheese, limited availability of ruminant source and its high cost as well as religious concerns, diet (vegetarianism), or ban on recombinant calf rennet in certain parts of the world have led to the focus of research on alternate sources for a satisfactory replacement for calf rennet. Elevated ratio of milk clotting to proteolytic activity (PA) that is, milk clotting index (MCI) at pH and temperature of cheese making and adequate thermolability to ensure whey products

without remnants of active coagulant are two specific calf rennet properties which should be mimicked by the substitute.[65]

Proteases from microbial and plant sources are being explored for their use in cheese production. Cheeses produced from these sources are acceptable to lacto-vegetarians. Limitation of most vegetable coagulants is their excessive PA leading to lower cheese yield and defects in flavor and texture.[83] The search for new potential plant coagulants is in continuous process in order to make them industrially useful and support the increasing global demand for diversified and high quality cheeses with new textures, tastes, and aromas for specific markets. There are also a few recent reviews on this topic.[65,126] After a brief introduction to milk clotting process and animal/microbial sources of milk-clotting enzymes, this chapter will focus on recent research findings on plant-derived proteases as milk coagulants along with characteristics of cheese produced by them.

1.2 MILK COAGULATION

Milk consists of casein, whey protein, and non-protein nitrogen. Approximately 3.0–3.5% of normal bovine milk is made up of protein and casein contributes around 80% of total protein. It is heterogeneous in nature and is composed of α_1-, α_2-, β, & κ-caseins constituting ~38, 10, 35, and 15 g per 100g of the total casein, respectively.[77] Individual caseins differ in their phosphate content and calcium-binding properties. Generally, α_1-, α_2-, and β-caseins bind calcium strongly and precipitate at relatively low calcium concentrations. In contrast, κ-casein is not sensitive to these calcium concentrations. κ-casein, in particular, has been proposed as a stabilizing factor which is otherwise insoluble in milk serum by promoting the formation of aggregates known as casein micelles.[77] Proposed models of micellar form conclusively state that calcium-sensitive caseins (α and β) are present in the interior of the micelle and calcium insensitive κ-casein on the outer surface providing stability and solvency to the casein micelle in native milk.[77] κ-casein helps in solubilizing the other three caseins, which are otherwise insoluble in milk serum, by promoting the formation of aggregates known as casein micelles.[38] Along with the casein proteins, important milk minerals, such as calcium and phosphorous also get bound inside the micelle.

Enzymatic cleavage of κ-casein, micellar aggregation or flocculation and gel formation are the steps involved in renneting reaction during cheese production.[77] Among these, enzymatic step is considered rate limiting in the process of milk coagulation. Proteases catalyzing the first step are

referred as milk coagulants. Based on the active site amino acid and catalytic mechanisms, proteases are grouped in six subclasses: aspartic proteases (AP), cysteine proteases (CP), serine proteases (SP), metalloproteases, threonine proteases, and unknown type proteases.[91,159] Although all proteases can cleave caseins, a protease with specificity for κ-casein at Phe^{105}–Met^{106} releasing hydrophilic caseinomacropeptide and hydrophobic para κ-casein as the products and a low PA toward other peptide bonds is considered to be the most efficient for cheese industry.[9] Majority of milk-clotting enzymes belong to AP, although a few enzymes from other groups have also been reported to possess milk-clotting activity (MCA).[126] Release of caseinomacropeptide into the whey results in the loss of negatively charged group and decrease in stearic stabilization. Hydrolysis of ~85% κ-casein reduces the colloidal stability for spontaneous aggregation of calcium-sensitive caseins. Subsequently, gel is formed as molecular chains connected through hydrophobic bonds via calcium cross linking. Whey is expelled from the casein network by synereses and the aggregated clot is then ripened to give rise to the final cheese.[53]

MCA is a measure of the end point of enzymatic hydrolysis and beginning of aggregation step in the process of milk coagulation. Soxhlet and Berridge methods are generally used and the latter is internationally accepted and recently revised.[64,65] Various methods have also been proposed based on different physical properties such as viscosity or conductivity which have the disadvantage for online monitoring.[55] Other methods, which can be used for continuous monitoring and are nondestructive in nature, are based on optical properties such as refraction, reflection, absorbance, or scattering. A fluorescence resonance energy transfer assay was developed recently to understand the enzymatic step independent of the aggregation step using the 98–108 sequence (bovine κ-casein)-derived peptide substrate.[66]

1.3 ANIMAL RENNETS

1.3.1 SOURCES

Animal rennet can be defined as an extract of the inner mucosa of the fourth stomach chamber, known as abomasum, of a calf or of the stomach of certain other young animals. Cow/buffalo/camel (calf), sheep (lamb), and caprine/goat (kid) are known sources of rennet. Initially, bovine (cow/buffalo) rennets gained industrial importance due to their specific properties such as MCI at specific pH and temperature as per cheese-making requirement.

Additionally, their thermolability ensured whey products without remnants of active coagulant.[65] Camel rennet (an acid protease) is yet another suitable milk coagulant which has been recently reported for the production of cheese with similar sensory characteristics to that by bovine rennet. Food and Agriculture Organization of the United Nations in Tunisia and Saudi Arabia established use of camel milk and its rennet in cheese production with variations in starter culture and calcium content to improve the nutritional status of African population.[48,118] Though lamb and goat rennet are comparable with calf rennet, they are found to be best suited for clotting milk of their own species.[49] Rennet paste is used in the manufacture of Southern Italian cheeses and those from Protected Designation of Origin (PDO). The paste is prepared by maceration and drying of stomach of recently suckled calves, lamb, or kid. This preparation, with inherently present or supplementary lipase, imparts characteristic flavor to the cheese.[58] Adult bovine rennets are not preferred in cheese making due to their higher pH sensitivity and general PA.

1.3.2 COMPOSITION OF RENNET

Milk-clotting enzymes generally belong to the class of APs, the great majority of which are optimally active at acidic pH. Chymosin and pepsin are the primary proteases of animal rennet and their ratio varies with age and precedent diet of the slaughtered animals. Ratio of chymosin to pepsin in calf rennet was found up to 95% in unweaned bovine calf. Camel rennet demonstrated high MCI, ~7 fold increase, as compared to bovine rennet.[67] Even with 85% sequence identity, camel chymosin exhibits 70% higher MCA than bovine chymosin toward bovine milk. These enzymes bring about specific Phe^{105}–Met^{106} bond cleavage of the bovine κ-casein.[76] Chymosin is highly specific for cleaving the caseinomacropeptide from κ-casein. This activates the disruption of the casein micelles subsequently inducing milk clotting.[37,61] Bovine rennet consists of three types of chymosin (A, B, and C) which differ from each other in their amino acid sequence. Existence of a fourth form of prochymosin, chymosin D, has also been reported which exhibited difference in electrophoretic mobility compared to other forms.[17,18] Specificity of pepsin is lesser compared to chymosin and hydrolyzes bonds with Leu, Phe, Tyr, or Val residues.[7,108] Calf rennet and adult bovine rennet additionally contain an aspartic protease, gastricsin, which constitutes 1–5% of the total enzymes, respectively.[141] Lamb and kid rennet contain lipases in addition to proteases which account for the organoleptical characteristics

of cheese produced by them.[4] During commercial manufacture of bovine rennet, zymogenic forms of proteases are converted to active enzymes in an activation step at acidic pH. Whereas, zymogens of goat and lamb do not require this step as they are converted to active enzymes automatically under the pH conditions of abomasa.[3]

Animal rennet generally shows high specificity toward κ-casein. However, depending upon the source of rennet, α- and β-caseins are also hydrolyzed at several sites. Lamb rennet exhibits a higher specificity to α-casein, especially α_1, along with extensive lipolysis.[63] Kid rennet is associated with high proteolysis during cheese preparation from milk of its own species. Further, unlike other animal rennets, camel rennet activity advances with age of the animal. κ-casein hydrolysis is similar to that of other animal rennets. However, when camel milk was used for cheese preparation, hydrolysis of α was quicker than β-casein.[117] Hydrolysis of α-casein was considerably reduced when cow's milk was substituted for camel milk. Further, flocculation time was less in comparison with other rennets. Fat recovery and cheese yield is similar to that by commercial bovine rennet.[100] These unique properties make camel chymosin's application in cheese production even from bovine milk.[155] Table 1.1 is a collation of characteristics of different animal rennets.

1.3.3 GENETICALLY ENGINEERED CHYMOSIN

With an enormous increase in global cheese production, limited availability of calf stomachs and ethical issues concerning animal slaughter, recombinant chymosin are now widely used for cheese manufacture in many countries. Further, as chymosin is produced by fermentation in genetically engineered microorganisms and not from abomasum of young animals, ethical issues associated with animal rennet does not arise for cheese consumption. Presently, it is estimated that fermentation-produced chymosin (FPC) has a global market share of about 80%.[65] FPC production involves isolation of mRNA and its cDNA synthesis by reverse transcriptase. cDNA is often used to clone eukaryotic genes in prokaryotes, as it is devoid of introns present in eukaryotic genes. Purified DNA is inserted into plasmid which is then introduced in the target microbes.[99] Yeast, bacteria, and fungi serve as host organisms for recombinant enzyme expression and large-scale production of chymosin.

TABLE 1.1 Characteristics of Different Animal Rennets.

Parameters	Bovine rennet	Lamb rennet	Kid rennet	Camel rennet	Refs.
Molecular weight of chymosin	35–40 kDa	36 kDa	36 kDa	40 kDa	Ref.[21,50,67,70,124]
Casein specificity	κ	κ, α_1	κ, α & β	κ	
Milk specificity	Bovine milk	Sheep milk	Goat milk	Bovine and camel milk	
Sensory attributes	Soft, firm cheese	Hard, elastic cheese with piquant taste & pungent flavor	Semi hard cheese with distinctive flavor	Soft firm cheese	

The recombinant enzyme has been extensively characterized and shown to be chemically and functionally identical to the chymosin. Further, the absence of bovine pepsin (always present at variable levels in traditional calf rennets) helps to improve cheese yield. There is no significant difference in yield and quality of cheese produced by FPC when compared to that by calf rennet. FPC expressed in *E. coli* was the first bioengineered enzyme to be approved as granted generally recognized as safe (GRAS) status by FDA in 1990. In *E. coli,* the recombinant proteins are not secreted but frequently synthesized as intracellular inclusion bodies and leads to considerable increase in process cost. *E. coli* is also not commonly accepted as safe for human consumption.[79] Presently, *Aspergillus niger* var. *awamori and Kluyveromyces lactis* are used for commercial production of bovine chymosin B and sold under the brand name of Chymax (Chr. Hansen, Denmark) and Maxiren (DSM Food Specialties, Denmark), respectively.

More recently, Chr. Hansen has introduced a new second generation FPC, Chy-Max M, which is fermentation-produced camel chymosin. This novel coagulant has some very interesting properties such as higher milk coagulation to general proteolysis ratio on *bovine* milk compared to bovine chymosin.[69] Recombinant chymosin of other animals have been attempted to improve their MCI and the yield of cheese produced. Recombinant lamb chymosin expressed in *E. coli* possessed better quality and coagulation properties as compared to FPC. They are found to have temperature instability above 45ºC that could be advantageous in making hard cheeses.[121] Caprine (*Capra hircus)* preprochymosin was cloned, characterized, and expressed in *K. lactis* and *S. cerevisiae.* Culture supernatants of both yeast transformants

showed cleavage of κ-casein and MCA after activation at acidic pH and revealed suitability of caprine chymosin as an alternative milk coagulant in cheese making.[152] Expression of active chymosin from buffalo (*Bubalus arnee bubalis*) in *Pichia pastoris* for the first time, demonstrated a higher κ-casein affinity in comparison to chymosin obtained through conventional methods. The recombinant chymosin thus produced does not require further *in vitro* activation process, making it yet another source of rennet for cheese manufacture.[150] Although FPCs are from the biotechnology industry and are expressed in genetically modified microorganisms (GMOs), they are products of GMOs and do not contain any living genetically engineered organisms (http://www.cheesescience.net/2008/07/what-is-fermentation-produced-chymosin.html).

There are a few reports on the expression of chymosin using plants as host organisms. Leaves of tobacco and potato and seeds of *Brassica napus* and Flax (*Linum usitatissimum*) have been used as host organisms to express chymosin. It was noted that leaves of tobacco and potato plants expressed chymosin at level of 0.1–0.5% of total soluble protein,[75] whereas seeds of transgenic plants accumulated up to levels of at least 0.5% (w/w) of total seed protein.[151] Although extensive research is being conducted on plant seeds for recombinant protein production (industrial and pharmaceutical use), there are still many technical challenges that have to be looked into before these systems can be considered as suitable and viable alternatives for large-scale production of recombinant proteins. Progress in last few years has led some pharmaceutically relevant plant-derived proteins (antibodies, vaccines, human blood products, growth regulators etc.) in reaching preclinical or commercial developmental phases.[25,68] However, large-scale production of recombinant chymosin using plants as bioreactors is yet to be explored.

1.3.4 MICROBIAL RENNET

Many microorganisms are known to produce extra cellular proteases that exhibit MCA. They are a promising source of milk coagulant due to their cheap production cost, greater biochemical diversity, and easy genetic modification possibility.[74,102] However, microbial coagulants are associated with high PA that leads to low yield and bitter taste of cheese produced.[65] Fungal sources are preferred for production of milk-clotting proteases. There are also a few reports on the production of milk-clotting proteases by bacterial species. Some bacterial species that are explored as putative sources for milk-clotting potential are *Bacillus subtilis natto*, *B. amyloliquefaciens*

JNU002, *B. amyloliquefaciens* D4, *B. subtalis*, *B. licheniformis*, *Nocardiopsis. sp*, *Enterococcus faecalis,* and *Myxococcus xanthus* strain 422.[85] Fungal rennet production is either by surface culture or submerged culture fermentation followed by purification to remove any other non-proteolytic enzymes.[65] These enzymes have molecular weights in a range of 30–45 kDa and contain one or two conserved aspartic acid residues at the active site. *Rhizomucor miehei* produces proteases that are similar in structure to bovine chymosin. Various cultivating conditions, media composition as well as reactor configuration, have been used for production of these proteases.

Commercial large-scale production of microbial (fungal) rennet (AP) has been established from *R. miehei*, *R. pusillus and Cryphonectria parasitica.*[58] High thermostability of fungal proteases make them remain intact even after maturation of cheese causing textural and flavor changes in the cheese produced. However, they are found acceptable in the production of Emmental and Italian-style cheese as they are processed at high temperatures.[58] Novel methods, like use of chemicals and/or by genetic mutations, have been used to decrease the heat stability of *Rhizomucor* species. Methionine oxidation with hydrogen peroxide or dye-sensitized photo-oxidation decreased the thermal stability of the AP of *Rhizomucor* species without significantly reducing their MCA.[35] Fungal AP with decreased thermostability has been generated through a potentially viable mutagenic approach. The single and double amino acid mutants of *R. pusillus* for AP displayed significantly lower thermostability without altering the enzymatic activity or its MCI. However, effect of mutations at different amino acid locations in *R. miehei* revealed higher MCI with certain mutations leading to even an increase in cheese yield.[85] Acylation of AP from *R. pusillus* with various acid anhydrides independently and in combination enhanced its MCA and MCI.[36] Some commercially available microbial rennets are (a) Fromase, Hannilase, Marzyme, Meito Rennet Super (MRS), Milase XQL/TQL/Premium, Enzymaks, Reniplus, Microclerici (*R. miehei*); (b) Suparen and Thermolase (*C. parasitica*); (c) Meito Microbial Rennet (*R. pusillus/miehei*) etc.[85]

Based on increasing specificity, milk coagulants may be placed in the following order: *C. parasitica* < *R. miehei* < bovine pepsin < FPC (bovine) and calf chymosin< FPC (camel).[85]

1.4 PLANT-DERIVED COAGULANTS

Plant extracts have been used as coagulants since ancient times although relatively little was known about their precise mechanism of action. The

earliest indication of cheese making using vegetable milk coagulating preparations descends from cave paintings around 5000 BC. Use of plant coagulants for cheese production increases the acceptability by the vegetarian population and further has an advantage of improving their nutritional intake.[136] However, many of them possess very low MCI, due to elevated PA, leading to development of off flavor, extensive digestion of curd, impaired taste, and pasty-bodied cheese which are considered unsatisfactory for commercial production.[122] Plant coagulants have been extensively used in Southern European and West African countries for traditional preparation of cheese. Spain and Portugal have the largest cheese varieties using aqueous extracts Cynara *sp.* as the vegetable coagulant.[122] In spite of high PA associated with *Cynara*, it has been well received commercially as rennin substitute. Different cheese varieties produced from Cynara *sp* include Serra and Serpa, Los Pedroches, La Serena and Torta del Casar cheeses (ewes' milk), Los Ibores cheese (goats' milk), and Flor de Guía cheese (ewes' and cows' milk mixture).[126] In rural areas of Sudan, dairy farmers use the berries of *Solanum dubium* to make white soft cheese using goat and sheep milk.[98] In West African countries like Nigeria and the Republic of Benin, leaf extracts from *Calotropis procera* (Sodom apple) have been used in traditional cheese making which has a distinctive soft creamy consistency and fine flavor , occasionally faintly bitter but piquant with advance ripening.[122]

Papaya (papain), pineapple (bromelain), fig (ficin, cucumisin), rice seeds (oryzasin), and sodom apple (procerain) are some examples explored for cheese production in various parts of the world. The search for novel plant based milk-clotting enzymes is still on to meet the rising global demand for cheese of diverse variety with enhanced quality.

1.4.1 SOURCES AND NATURE OF MILK-CLOTTING PLANT PROTEASES

Proteolytic enzymes are present in almost all kinds of plant species and their tissues and it appears to be a general rule that all proteolytic enzymes have the ability to clot milk under appropriate conditions.[144] Milk-clotting proteases have been characterized and purified from a variety of tissues such as seeds, flowers, leaves, roots, and stem as well as from the latex of several plants from Asteraceae, Caricaceae, Moraceae, Asclepiadaccae, Apocynaceae, and Euphorbiaceae families.[42] Latex has an added advantage over other plant parts due to excess amount of proteases present in it.[24]

Milk coagulating plant proteases reported till date are endopeptidases from Aspartic, Cysteine, and/or Serine subclasses.[29] These subclasses are categorized based on inhibition by specific chemicals. AP are characterized by two aspartic residues at catalytic site and are specifically inhibited by pepstatin.[56] They have gained attention in cheese industry due to their similarity in kinetic parameters/substrate specificity with that of chymosin. CP (thiol protease) possess cysteine at its catalytic site and are inhibited by iodoacetic acid.[56] One of the largest groups of proteases, found in eukaryotes and prokaryotes are SP.[56] They possess serine residue in their active site and are inhibited by phenyl methyl sulfonyl fluoride (PMSF). SP and CP are catalytically very different from AP in that the nucleophile of the catalytic site is part of an amino acid, whereas it is an activated water molecule in the latter.[56]

Ample variety of plant species contains AP, collectively named as Phytepsins (EC 3.4.23.40). Thistle flowers from several species of Cardueae tribe (Asteraceae) have been the subject of intensive study due to the presence of AP with extremely high specific activity.[31,130] However, AP from fruits of *Balanites aegyptiaca* has also been reported.[23] Plant latex proteases generally belong to cysteine family and possess intense PA in crude form.[16] Nevertheless, some latex milk-clotting proteases also fall under serine subclass. SP are almost present in all plant parts and several vegetable milk coagulants identified and characterized belong to this super family.[56] Table 1.2 represents a compilation of potential vegetable milk coagulants purified and characterized from different plant sources. Further, crude proteases with milk-clotting potential from many other plant sources have also been reported. As these proteases have not been purified, they are not included in Table 1.2.

TABLE 1.2 Potential Vegetable Milk Coagulants Purified and Characterized from Different Plant Sources.

Plant part	Plant source	Family	Name of protease	Type of protease	Ref.
Latex	*Calotropis gigantea*	Asclepiadaccae	Calotropin FI, FII, D1 and D2	Cysteine	Ref.[2,107]
	Asclepias curassavica	Asclepiadaccae	Asclepian	Cysteine	Ref.[80]
	Funastrum clausum	Apocynaceae	Funastrain	Cysteine	Ref.[101]
	Calotropis procera	Asclepiadaccae	Procerain	Cysteine	Ref.[71]

TABLE 1.2 *(Continued)*

Plant part	Plant source	Family	Name of protease	Type of protease	Ref.
	Philibertia gilliesii	Apocynaceae	Philibertain	Cysteine	Ref.[125]
	Ficus carica	Moraceae	Ficin	Serine	Ref.[156]
	Ficus religiosa	Moraceae	Religiosin, Religiosin B, Religiosin C	Serine	Ref.[72,73,127]
	Streblus asper	Moraceae	Streblin	Serine	Ref.[146]
	Euphorbia neriifolia	Euphorbiaceae	Neriifolin, Neriifolin S	Serine	Ref.[158]
	Euphorbia nivulia	Euphorbiaceae	Nevulian	Cysteine	Ref.[20]
	Euphorbia prunifolia	Euphorbiaceae	Prunifoline	Serine	Ref.[89]
	Euphorbia microsciadia	Euphorbiaceae	Microsciadin	Cysteine	Ref.[120]
Flowers	*Cynara cardunculus*	Asteraceae-Cardueae	Cardosin, cyprosins	Aspartic	Ref.[87]
	Onopordum acanthium	Asteraceae	Onopordosin	Aspartic	Ref.[30]
	Cirsium vulgare	Asteraceae	Procirsin	Aspartic	Ref.[86]
	Centaurea calcitrapa	Asteraceae	N/A	Aspartic	Ref.[40]
	Silybum marianum	Asteraceae	N/A	Aspartic	Ref.[149]
Inflorescence	*Cynara scolymus*	Asteraceae	Cynarase	Aspartic	Ref.[81]
Leaves	*Lactuca sativa*	Asteraceae	Lettucine	Serine	Ref.[83]
	Cynara scolymus	Asteraceae	Cynarase	Aspartic	Ref.[81]
Fruit	*Bromelia balansae*	Bromeliaceae	Balansain	Cysteine	Ref.[110]
	Actinidia chinensis	Actinidiaceae	Actinidin	Cysteine	Ref.[12]
	Maclura pomifera	Moraceae	Macluralisin	Serine	Ref.[123]
	Cucumis melo	Cucurbitoideae	Cucumisin	Serine	Ref.[147]
	Balanites aegyptiaca	Zygophyllaceae	N/A	Aspartic, Serine	Ref.[23]
	Bromelia hieronymi	Bromeliaceae	Hieronymain 1 Hieronymain 2	Cysteine	Ref.[27,29]

TABLE 1.2 *(Continued)*

Plant part	Plant source	Family	Name of protease	Type of protease	Ref.
Seeds	*Onopordum turcicum*	Asteraceae	N/A	Aspartic	Ref.[143]
	Solanum dubium	Solanaceae	Dubiumin	Serine	Ref.[8]
Root	*Cynara scolymus*	Asteraceae	Cynarase	Aspartic	Ref.[81]
Rhizome	*Zingiber officinale Roscoe*	Zingiberaceae	Zingibain	Cysteine	Ref.[139]

1.4.2 HIGHLIGHTS OF RESEARCH ON VEGETABLE MILK COAGULANTS

Genus *Cynara* L. belonging to Asteraceae-Cardueae family, native of Mediterranean countries particularly Spain and Portugal, have been extensively used for production of traditional cheese from ewe's milk. Aqueous flower extracts from Cynara *sp.* cleave bovine κ-casein at Phe^{105}–Met^{106} similar to chymosin, however exhibits broader specificity as it hydrolyzes other peptide bonds in α- and β-caseins. This high PA contributes to bitterness and slight astringent taste of cheese that disappear during maturation. This was found to contribute a specific flavor for cheese produced from Cynara *sp*.[122] Two AP, Cardosin A and B, have been characterized in *C. cardunculus* flower extracts. They differ in their amino acid sequences and are probably the products of two distinct genes.[153] Cardosin A bears similarity to chymosin in its activity and specificity and Cardosin B to that of pepsin.[138] *C. scolymus,* generally used as a vegetable, is also found to possess three milk-clotting AP, Cynarase A, B, and C that may find value in the dairy industry in the same way as that of *C. cardunculus.*[128] Quality and activity of proteases from *C. cardunculus* depended strongly on both environment and conservation conditions. Limited availability of resources greatly hinders large scale industrial production of cheese from them.[119] However, increased demand for vegetarian cheese in the market drives the need for plant milk coagulants.

Callus and cell suspension culture techniques were therefore implemented to produce milk-clotting proteases from *C. cardunculus.*[103] Development of recombinant proteases from such plants may also provide solution to these problems. Cardosin B from *C. cardunculus* was successfully expressed in *K. lactis*. This new vegetable coagulant resulted in higher cheese yield from cow, sheep, and goat's milk in comparison to calf rennet and is regarded as excellent

alternative to chymosin.[13] A standardized procedure for extraction of cyprosins from *C. cardunculus* flowers has been reported by Fytozimus Biotech, Inc.[95] Commercially available proteases from *C. cardunculus,* Cynzime (cyprosins), Cyprozime (recombinant cyprosins enzyme), and Cynmix (mix of cynzime and cyprozime) are manufactured and marketed by Fytozimus biotech, Canada.

Acceptability of vegetable coagulant from Cynara has led to extensive research to identify proteases from diverse plant parts of other genera. Purified AP from *O. turcicum* flowers was found suitable as milk coagulant in spite of its *caseinolytic activity* (CA) being five times more than that of bovine rennet.[143] Two purified proteases from aqueous extracts of *Centurea calcitrapa* flower were shown to hydrolyze bovine, ovine, and caprine milk caseins but found appropriate to be used in dairy industry to produce cheese only from ovine and caprine milk.[145] A purified endopeptidase, Onopordosin from *O. acanthium* (Asteraceae) has been proved to be an effective vegetable coagulant at acidic pH to produce cheese from pasteurized bovine milk.[30] An active AP precursor from *C. vulgare* (Asteraceae), Procirsin, has been cloned, characterized, and expressed in *E. coli*. This AP showed a high specificity toward κ-casein that could effectively be used as rennet alternative.[86] The cloned aspartic protease mimicking chymosin in its mode of action was acquired from floral extracts of *Scolymus maculatus*.[96] Floral extracts of *Citrus aurantium* exhibited MCA over a wide range of pH ascertaining its AP suitability as plant rennet.[92] Partially purified and characterized flower extracts of *S. marianum* (Asteraceae) and *Moringa oleifera* (Moringaceae) as well as crude extracts of *Arctium minus* (Asteraceae) exhibited significantly high MCI for a suitable rennet substitution.[34,115,149] SP and CP in crude floral extracts of *C. gigantea* (Asclepiadaccae) were also reported to clot milk.[15]

Leaves of *C. procera* are being used in traditional preparation of wara cheese in West African countries.[62] Extensive studies on scientific validation and specific mechanism of action in milk coagulation has been carried out to produce cheese with acceptable quality.[10,19] Due to inherent toxicity associated with *C. procera*, commercial production has still not been approved by regulatory agencies. Similar to leaf extracts of *C. procera, Cymbopogon citratus* leaf extracts were also found to be effective in the production of wara cheese.[5] Crude extracts from leaves of *F. bengalensis*, *F. elastica*, *Carica papaya*, *S. asper,* and *C. gigantea* demonstrated to be good sources of plant milk coagulant.[15,32,39]

A CP from *C. procera* latex, Procerain B, has been reported to be a potential vegetable coagulant. It has been characterized, immobilized, sequenced, cloned, and modeled at molecular level.[131–133] Studies on Calotropins (FI and FII), proteases purified from latex of *C. gigantea,* confirmed Calotropin FI to

be a chymopapain like enzyme which induced milk clotting more effectively than Calotropin FII.[1] Further, crude enzymes of *C. gigantea, Allamanda cathartica, Plumeria rubra, Jatropha curcas,* and *Euphorbia antiquorum* latex were found to possess milk-clotting potential.[14,15] *Sideroxylon obtusifolium* crude latex extract possessed MCI similar to mucor rennin indicating its feasibility as a potential rennet substitute.[129] Other milk coagulating latex proteases studied include, SP: ficin (*F. carica*), religiosin & religiosin A (*F. religiosa*) and CP: papain (*C. papaya*), asclepain (*Asclepias speciose)* & nevulian (*E. nivulia*).[72,73,156,157]

Prunifoline, a purified SP from *E. prunifolia*, is a potential milk-clotting enzyme. It was active over a wide range of temperature and showed preferential substrate specificity toward casein that may find utility in food and other biotechnological industries.[89] A novel CP microsciadin, purified from *E. microsciadia* latex was found associated with high MCA and MCI comparable to calf rennet suggesting its potential in dairy industry.[120] Latex proteases from roots of *Jacaratia corumbensis* (both partially purified and purified) characterized as CP was found to be potential source of vegetable milk-clotting enzymes.[43] Latex from leaf petioles of *P. gilliesii* and fruits of *A. fruticosa* were also found suitable to be a source of vegetable coagulant.[109]

Even with limited research, stem of plants were also considered as good source of milk-clotting proteases. Accumulated data reveals the possible milk-coagulating potential associated with crude extracts of *Opuntia phylloclades, Cereus triangularis, Aloe.l. sp*[39] and *C. procera.*[105] Yet another study sheds light on casein subunit specificity and milk-clotting potential by SP and CP from *C. gigantea* stem extract (crude) and their similarity with that of commercial rennet.[15]

Fruit extracts of plants are another excellent source of vegetable coagulants. Purified proteases from *A. chinensis* (actinidin) *B. balansae* (balansain), and *B. hieronymi* (hieronymain) are a few examples of fruit-derived vegetable coagulants.[12,27,110] Two milk-clotting proteases, an aspartic and a serine, were characterized from *B. aegyptiaca*. Biochemical and rheological characterization of a protease from fruit extracts of *Withania coagulans* ascertained its milk-clotting potential.[22] Partially purified protease from *S. dubium* fruits also proved to be an effective source of plant rennet which sustained its activity when stored at refrigerated temperature.[47] Crude extracts of kiwi (*A. deliciosa*) and melon (*C. melo*) fruit pulp were reported to possess milk-clotting potential. However, kiwi extract exhibited slightly higher PA and similar cheese yield to that of chymosin.[92] Purified protease, Zingibain, from rhizome of *Z. officinale* is yet another coagulant which is effective on milk from different animal sources despite different cheese characteristics.[54]

Use of seed, as milk coagulant source, generally allows the continuous availability for industry. Dubiumin, a SP purified from *S. dubium* (Solanaceae) seeds possess high MCI in comparison to other plant coagulants and stable over broad range of pH, temperature, and denaturants.[8] Crude extracts from seeds of *Albizia lebbeck* demonstrated 15 times higher milk-clotting potential than *Helianthus annuus* with a PA satisfactory for cheese ripening.[44] Purified SP from *B. napus* seeds showed unique physicochemical characteristics with high specific MCA demonstrating its suitability as an economic and promising rennet alternative.[46] Partially purified *M. oleifera* seed proteases exhibited MCI even greater than that of calf rennet proving its appropriateness as potential vegetable coagulant.[142]

In vitro production of plant proteases through micro propagation, cell suspension, and callus production, as suitable source of vegetable coagulant has also been experimented. *C. calcitrapa*, an AP with good MCA, was produced by cell suspension and hairy root culture. AP from *S. marianum,* CP from *F. carica* and protease from *S. dubium* were produced via methods such as micro propagation, cell suspension and immobilized cells using callus cultures. These were suggested as reliable alternative source for vegetable milk coagulant production. *In vitro* production has an advantage of producing enzymes that are free from pathogens and reducing the time period of cultivation of plantlets. This warrants possibility of industrial production of these proteases for their use in cheese manufacture.[56]

1.5 CHARACTERISTICS OF CHEESE

Cheese quality is best defined as degree of acceptability of the product by the end user.[112] Many varieties are produced from a limited range of raw materials, which include milk, lactic acid bacteria, milk coagulant, and salt. Yet "there is taste preference for every cheese and cheese for every taste preference" as quoted by Olson.[104] Based on the method of coagulation, it can be classified into three groups, that is, acid-coagulated, acid- & heat-coagulated, and rennet-coagulated. The last group can be subdivided into various classes depending on the technological process and ripening. Rennin-coagulated cheese production represents 75% of total cheese production.[52] Quality criteria for cheese involve different characteristics like:

- Sensory (taste, texture etc.),
- Physical (sliceability, mouth feel etc.),
- Method of cooking (ease of flow, browning),

- Nutritional/composition (proteins, fatty acids etc.),
- Chemical (casein, free fatty acids, etc.) and
- Safety (absence of pathogenic organisms and poisonous compounds).

Analyzing cheese composition, flavor and ripening changes involves sample isolation or fractionation methods, chemical and enzymatic assays and instrumental methods.[57,140] While some are well established standard methods, some still need to be researched and improved.[140] Unlike many processed food products, where stability is the crucial criterion, cheese is a biochemically dynamic product which undergoes significant changes during ripening period.[94] Many freshly made cheese varieties are bland with largely similar flavors. Characteristic aromas are gained during ripening process due to the production of specific volatile and non-volatile organics. In addition, some sapid compounds are involved in the basic cheese taste; they include organic acids (lactic and succinic acids), amino acids (glutamic acid) and minerals.[26] The primary biochemical pathways during cheese ripening include proteolysis, lipolysis, residual lactose, lactate, and citrate metabolism (starter culture fermentation). Subsequent secondary biochemical processes that follow are important for the development of volatile flavor compounds (Fig. 1.1).

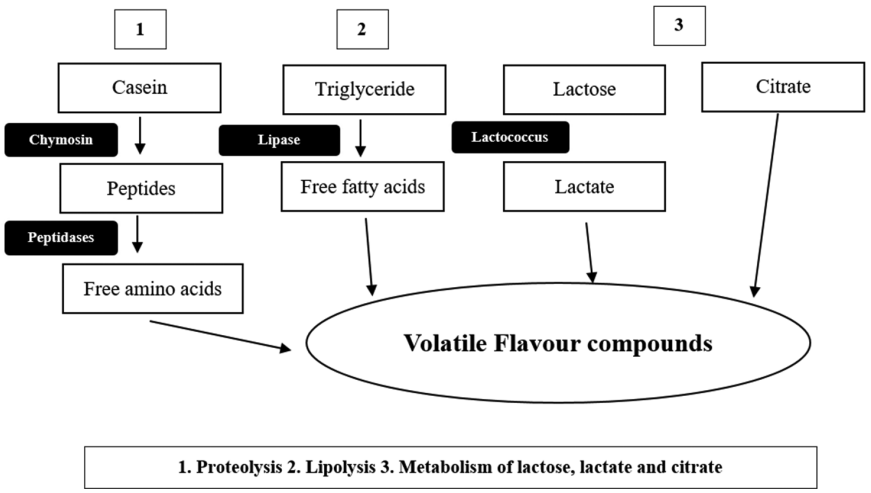

FIGURE 1.1 General biochemistry of cheese ripening. Adapted from McSweeny and Sousa.[94]

In standard cheese preparations, lipolysis occurs in all types of cheese by endogenous/exogenous lipases. Lipases in cheese originate from milk, rennet preparation (rennet paste), starter bacteria and if used, externally added lipases.[59] Milk lipoprotein lipase is protected in milk fat globular membrane (MFGM). During cheese manufacture, this protection is impaired due to the damage to MFGM leading to fat hydrolysis.[93]

1.5.1 SUBUNIT SPECIFIC CASE IN HYDROLYSIS BY VEGETABLE COAGULANTS

Milk coagulants have two roles in cheese making: to coagulate the milk as well as to contribute to the development of the sensory characteristics of the cheese due to the variable amount of the enzymes retained in the curd.[57] The percentage of residual chymosin depends on enzyme type, pH, and cooking temperature.[148] The ~30% of the total enzyme activity remains as residual chymosin after milk clotting. This is responsible for proteolysis in many varieties of cheese during their ripening. Primary proteolysis is caused mainly by residual rennet (or any other milk coagulant) leading to the formation of large to medium-sized peptides from caseins which are further degraded into small peptides and eventually to free amino acids in a process generally known in whole as secondary proteolysis. Primary proteolysis has a role to play in development of cheese texture, whereas secondary proteolysis is implicated with cheese flavor.[154] Different casein subunits are reported to be responsible for distinct cheese properties. α (α_1 and α_2), β- and κ-caseins are found to be associated with cheese texture, bitterness, and milk-clotting efficiency, respectively.[154]

Rate of hydrolysis of caseins influences the yield, consistency, as well as flavor of cheese. Slow degradation of α- and β-caseins is preferred for the production of a firm curd, which occurs with chymosin as coagulant.[28,51] During ripening, casein subunits are primarily cleaved at specific sites (α_1 at Phe_{23}–Phe_{24} and β at Leu_{192}–Tyr_{193}) by chymosin; however, α_2 is resistant to its hydrolysis. Extent and pattern of proteolysis considerably varies based on manufacturing practices, ripening protocols, and is unique for a particular type of cheese.[93]

It is of great importance to evaluate degradation patterns of the caseins by potential plant milk coagulants because of their effects on yield, consistency, and flavor of the mature cheese. Further, their strong PA leads to extensive breakdown of caseins in the cheese matrix resulting in distinct features of the final product.[88] Polyacrylamide gel electrophoresis (urea and tricine) as

well as HPLC have been used as analytical tools for the separation, identification, and quantification of caseins hydrolysate constituents.[44,111] PAGE analysis exhibited a complex hydrolytic pattern of α_1- and β-caseins with Cardosins from *C. cardunculus* (Cardoon), used for cheese production from ewe's milk, as compared to that by chymosin. They even targeted two peptide bonds of α_2-casein. The bitter taste associated with resulting cheese using these coagulants may be contributed by such low molecular weight peptides.[31,88]

Cynarase A, B, and C (proteases from *C. scolymus*) hydrolyze other bonds on κ-casein in addition to Phe[105]–Met[106,] [82] Cynarase A and C additionally hydrolyze α- and β-casein at length.[33] Studies on La Serena, a semi hard cheese manufactured from ewe's milk using Cynara proteases, also revealed this degradation pattern endorsing the reason behind the characteristic flavor and texture of cheese made from Cynara *sp.* However, peptidases and proteases produced by microflora in ewe's milk furthermore contributes to casein hydrolysis.[134,135,137] Use of Cynara proteases for cheese production from bovine milk sometimes resulted in inferior quality cheese, principally in texture and flavor due to excessive proteolysis.[126] Considerable research has gone in to reduce the extent of proteolytic effect by Cynara species in cheese making using concentration of substrate proteins via ultrafiltration (UF).[6] Nevertheless, they are used widely in artisanal cheese production from ovine and/or caprine milk and are commercially accepted by the population for its characteristic taste and soft texture.[65,122]

Milk-clotting potential has been identified in various parts of other plants as well. It is essential to monitor proteolytic process, as a well-proportioned breakdown of caseins is important to guarantee desirable attributes in cheese. But, only very few studies have focused on this aspect. PAGE analysis of *C. calcitrapa* and *S. marianum* displayed hydrolysis of α- and β-casein similar to that of *C. cardunculus.*[41,149] Purified proteases from seeds of *A. lebbeck and H. annuus* revealed hydrolytic affinity toward all caseins and exhibited an order of hydrolysis: κ-CN ≥ α-CN ≥ β-CN. However*, H. annuus* proteases exhibited slower hydrolysis of α-CN and β-CN in comparison to *A. lebbeck* indicating the superiority of *H. annuus.*[44] Bovine casein hydrolysis by *S. dubium* seed protease revealed lower susceptibility of α-casein as compared to β-casein proposing its suitability in accelerating cheese ripening or as rennet substitute.[97]

A rapid hydrolysis of κ-casein and slow degradation of α- and β-caseins are essential requirements in cheese making which was observed on casein hydrolysis by hieronymain (*B. hieronymi)*, a cysteine protease. Miniature cheese prepared from it was also found to possess acceptable sensory

attributes.[28] Even with higher specificity toward β-casein as compared to κ-casein, Actinidin from *A. chinensis* could induce milk clotting.[114] Rapid and intense hydrolysis of α- and β-caseins with apparent disappearance of intact κ-casein was shown by fruit extracts of *W. coagulans*. This coagulant was suggested to be useful in accelerating cheese ripening which is otherwise a slow process.[113] Electrophoretic profiling of early stages of milk clotting by Onopordosin (*O. acanthium*) exhibited a degradation pattern analogous to that by bovine chymosin. Cheese manufactured using this protease produces semi-hard cheese with sensory acceptability score similar to commercial cheese with some differential characteristics.[30]

Effect of time on casein hydrolysis was accounted on different purified plant proteases like Philibertain, Balansain, Hieronymain, and Asclepian, each exhibiting different specificity for casein. Hieronymain exhibited affinity toward κ-casein with slow hydrolysis of other caseins while remaining three proteases showed no preference to any casein during first 24 h.[109] Similar study on crude proteases from different plant parts of *C. gigantea* revealed κ-casein specificity by latex, stem, leaf, and flower proteases during 1 h- and 24 h-incubation of enzyme with standard casein. Latex proteases exhibited complete hydrolysis of caseins whereas stem proteases exhibited a pattern similar to that by commercial rennin in 1 h-incubation. Complete hydrolysis was observed on 24 h-incubation by all proteases including commercial rennin. This study concluded suitability of *C. gigantea* stem proteases as potential vegetable coagulant.[15] An electrophoretic profiling of time-dependent degradation of caseins by purified flower proteases of *S. maculatus* also revealed similarities to that by calf rennet. κ-casein was degraded at first, followed by slow degradation of α-casein and then β. This characteristic profiling assures production of firm milk clots, a primary criterion for cheese making.[96]

1.5.2 ATTRIBUTES OF CHEESE PRODUCED BY PLANT COAGULANTS

To substantiate the cheese-making efficiency by vegetable coagulants, laboratory-level cheese preparation and further analyses were carried out. Cheese from bovine milk by leaf extracts of *C. procera* resulted in harder, less cohesive, and gummier cheese though the yield was slightly higher than calf rennet using direct acid hydrolysis.[19] When seed protease of *A. julibrissin* (Persian silk tree) was used, cheese prepared did not develop bitterness even after 3 months of ripening.[106] Comparative study on cheese

prepared by kiwi, melon, and ginger crude proteases with that by commercial rennin was performed in terms of temperature dependence, yield, and textural properties. The study revealed cheese from kiwi fruit extract to be similar in sensory properties to that by chymosin, whereas cheese from melon extract exhibited soft creamy texture.[92] *Z. officinale* has been tested for cheese production using different substrates. Cheese produced from buffalo milk as substrate had increased cohesiveness and smoothness during ripening with high overall acceptability score.[45] However, with camel milk, yield and overall acceptability score was lower than that by rennet.[160]

Cheese produced by crude latex extracts (*E. tirucalli, E. neriifolia, E. nivulia* and *Pedilanthus tithymaloides*) were reported to match with that by papain but differ considerably from commercial cheese in their nutritional quality.[90] Results from a study comparing cheeses from cow and sheep milk using three different local coagulants of plant origin (*C. procera* leaf, *C. papaya* leaf, and lemon fruit) revealed the suitability of sheep milk and *C. papaya* to be good substitutes to commonly used cow milk and *C. procera* extract. In terms of yield and dry matter, *C. papaya* competed favorably with local coagulant *C. procera*. Sheep milk additionally had favorable nutritive value and curdling time.[11]

Diverse factors can affect the quality of cheese produced using plant coagulants. Type of milk used as substrate is one such factor. Sheep's milk contains higher protein concentration than bovine milk. In order to improve quality of cheese from bovine milk using vegetable coagulants, techniques such as UF that manipulates the protein level in milk has been established. Bovine milk when modified to resemble sheep's milk by UF, improved quality of cheese.[6] Concentrated UF milk had an added advantage of decreasing the undesirable proteolysis which is inherent in the use of plant coagulants.[84] Study on aqueous extract of *C. cardunculus* (cardoon) supported this effect of UF on proteolysis. However, its sensory characteristics did not improve unless substantial quantity of regular cow's milk was added.[6] UF white brined-cheese is produced in some dairy plants in Iran from goat's milk using commercial chymosin. Though these cheeses are advantageous economically with high yield, they are not suitable organoleptically as they exhibit considerably slow proteolysis during ripening.[60] Proteases from fruits of *W. coagulans* enhanced the proteolysis during UF white brined-cheese ripening and could accelerate the ripening process improving their sensory attributes.[113] Composition (concentration of casein and fat), somatic cell count, enzymatic activity, pH etc. of milk are other factors that contribute to cheese quality.[78]

1.6 FUTURE PROSPECTS

In spite of all the data gathered on different aspects of milk-clotting plant proteases in the last few decades, there are several breaches in our knowledge regarding regulation of proteolysis and heterogeneity of these enzymes in each plant species to suit their role as rennet alternative. Plant protease activity and quality depends on environmental and cultivation conditions, which limits its large-scale production. Micro propagation and plant cell culture for production of proteases may overcome some of these limitations. Production of milk-clotting protease by designing recombinant systems via genetic approaches is the next line of advancement. Such an approach has been used in commercial production of Cyprozime from *C. cardunculus* by Fytozimus Biotech, Canada. This may help in overcoming the problems on coagulant performance due to climatic, seasonal, and other environmental changes. Diverse protocols are used for CA and MCA assessments of plant coagulants that make it difficult to compare plant coagulants' activity reported by different investigators. Thus, development of standard uniform protocols for both MCA and CA will help in identifying relative coagulation potentials of these proteases. Cheese ripening is an expensive, unpredictable, and slow process. Efforts are on to explore the high CA associated with plant coagulants to accelerate cheese ripening, while retaining or improving characteristic flavor and texture to reduce the time and costs of storage/maturation.[97,113] Recent rapid progress in some of the above-mentioned areas may lead to application of plant proteases within the foreseeable future.

1.7 CONCLUSIONS

Traditionally, cheese has been produced using calf rennin, a protease with specific properties. However, due to a steady increase in world cheese production, which escalated from over 5.5 million tons in 1962 to 20.7 million tons in 2012, available calf rennet is not able to meet this demand . This has led to intense research for rennet substitutes, and presently enzymes of microbial origin, recombinant proteases from GMOs, and plant proteases are also commercially used as milk coagulants. The generally used milk-clotting enzymes belong to the class of AP, which specifically cleave the bovine κ-casein Phe^{105}–Met^{106} bond. Milk-clotting proteases are found in many plant parts as well as in their latex. However, to be an effective rennin substitute, they should mimic calf rennin and exhibit a high ratio of clotting to PA at pH and temperature of cheese making. This chapter focused on

plant-based milk-coagulating proteases from different sources, their traditional use, specificity, and characteristics of cheese produced.

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KEYWORDS

- **abomasum**
- **AP**
- **bovine**
- **calf rennin**
- **camel**
- **casein**
- **cheese**
- **coagulant**
- **dairy**
- **fermentation**
- **flavor**
- **hydrolysis**
- **KID**
- **lamb**
- **microorganisms**
- **milk clotting**
- **pepsin**
- **plant-latex**
- **protease**
- **proteolysis**
- **recombinant**
- **ruminant**

- **texture**
- **thermolability**
- **vegetable coagulant**

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CHAPTER 2

RHEOLOGICAL CHARACTERIZATION OF FRUIT FILLINGS

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CONTENTS

ABSTRACT

Fruit preparations like fruit ripples, fruit fillings, fruit toppings, and fruit sauce represent intermediate products used in bakery product, fermented milk products. Rheological properties of fruit preparations are of prime interest in designing unit operations, process optimization, and quality assurance; also provides the information to discuss foods and their molecular structure, textural characteristics changes during process also the mathematical views for new product development. The sensory concepts such as mouthfeel and body could be linked up with consistency as well as more rheological properties referring to the texture characteristics of the products. The rheological properties of fruit ripple mostly get affected by the fruit content, type, and concentration of hydrocolloids, starches, sweetening agents, acidity regulators, and processing conditions. Hydrocolloids should be used in combination to get proper gel structure, heat stability, and structural stability. Native starch fails to withstand with high heat processing thus, should be chemically modified, and/or used in combination of hydrocolloids. Fruit ripples when added with hydrocolloids/starch act as non-Newtonian pseudoplastic fluid. Oscillatory tests can be used for determination of the viscoelastic properties such as G′ (storage modulus) and G″ (loss modulus). Herschel–Bulkley model can be fitted adequately with minimum standard errors.

2.1 INTRODUCTION

Fruits are being a part of human's food since immemorial times; those are being used for the preparation of jams and jellies as an art by the homemaker and confectioners.[18] Fruit preparations including fruit ripples (fruit fillings), fruit toppings, and fruit sauces represent intermediate products used in fermented milk products such as yogurt, sour milk and fresh cheese, and in pudding, cream, fruit milk, and ice cream. Fruit ripples or fruit fillings are also used in bakery and confectionery products.[37] Bakery fruit fillings are fruit preparations made from fruit-based raw material, sugar, water, and stabilizing/thickening agents which are used as a filling component in bakery products such as cakes, pastries, doughnuts, and so forth.[11] These preparations obtained from natural fruit purees represent a rich source of dietary fiber (mainly cellulose and pectin) and biologically active compounds (polyphenols, macro- and microelements, vitamins, etc.).[10] Nowadays the fastest growing segments of the food industry is bakery products commingled with

various fruit fillings, which are on high consumer demand for their fresh-fruity flavor and ready on-the-go snacks and meals.[11]

Fruit fillings are a little-studied product.[2] According to the *German Association for Food Law and Food Science* (BLL) definition, fruit preparations are "*products meant for the production of dairy products which, as a rule, are produced from fruits or fruit constituents and various sugars, and also essences, flavors, coloring foodstuffs, thickening agents and consumable acids, and which are preserved by appropriate methods. The BLL guideline also specifies the quality requirements for fruits and fruit constituents meant for processing.*[37]"

This chapter discusses the rheological aspects of a fruit ripple (filling).

2.2 RHEOLOGICAL ASPECTS OF A FRUIT RIPPLE

Rheological properties of fruit purees/fruit preparations are one of the prime interests in development of fruit products for technological and marketing reasons. The rheological characterization of food is important for designing unit operations, process optimization, and quality assurance,[4,17] also provides the information to discuss foods and their molecular structure, textural characteristics changes during process and also provides the mathematical views for new product development.[5,17] The rheological parameter is one of the useful tools for understanding changes in food structure during processing which helps to control the product quality.[12] To fulfill the consumer demands for processed foods with high quality, it is needed to define changes in rheological properties of foods during processing operations and storage that may affect their overall consumer acceptability.[5] The sensory attributes like mouthfeel and body can be linked up with consistency as well as more rheological properties referring to the texture characteristics of the products.[1,5]

2.2.1 FACTORS AFFECTING RHEOLOGY OF FRUIT RIPPLES

Fruit fillings/preparations/ripples usually consist of fruit pulp, fruit titbits, hydrocolloids, and/or starches as thickening agent, sweeteners, acidity regulator, water, flavors, and colors. The rheological properties of fruit ripple are mostly affected by the fruit content, type, and concentration of hydrocolloids, starches, sweetening agents, and acidity regulators. Moreover the flow properties are mainly affected by the processing conditions.[1,3,6,36] The

rheological parameters like flow behavior and yield stress are affected by the total fruit content in fruit ripples.[14]

2.2.1.1 EFFECT OF FRUIT QUANTITY ON FRUIT RIPPLES

The quantity of fruit in the fruit preparations generally amounts to 35%.[9] However, in case of raspberry, redcurrant, raspberry–blackberry, gooseberry, pineapple, and plum, the fruit content is at least 30%; and for banana and blackcurrant at least 25%. Coloring ingredients such as juices from grape, cherry, and red beet are not considered as a part of total fruit content. The dosage of fruit preparations in dairy products ranges between 5 and 25%, depending on the intended use. The minimal amount of fresh fruit in fruit preparation for yogurts is usually 6%.[37]

From the physical point of view, the fruit preparations are two-phase systems containing dispersed phase with solid particles of fruit pulp and colloidal continuous phase with sugars, salts, acid regulators, also fraction of soluble hydrocolloids/gums and starch dissolved in water.[30]

Type and variety of fruit, total moisture content of the fruit, pectin and fiber content majorly make impact on the rheology of the fruit preparations/ripples. Rheological characteristics obtained by fruit ripple with fruit pulp of one variety of fruit, can definitely differ in the rheological characteristics of the fruit ripple made with the different varieties of the same fruit.

Pulp concentration and particle size are one of the most important structural parameters having a large effect on the rheological properties of fruit ripples. Reduction in particle size and pulp content leads to decrease in yield stress and apparent viscosity. The larger values of indices, in the Herschel–Bulkley equation, are obtained by purees with high pulp content. Different effects in consistency and flow are obtained with different types of fruits, due to difference in quantity and proportions of the pectin fractions in those fruits.[16,17] Consistency index is found to decrease with the decrease in particle size of the puree. Yield stress of purees increases with the increase in pulp content, a small change in pulp content in fruit preparation strongly affect the values of yield stress.[12] Similar results were obtained by Maceiras et al.[24] and Nindo et al.[29] Fruit pulps (mango, apple, guava, tamarind, raspberry, apricot, pineapple, apricot, black currant, etc.) usually have a shear thinning nature. Consistency of the cream fillings/fruit filling with additives is greater as compared to that ripple, which only contains fruit pulp.[32]

2.2.1.2 EFFECT OF STABILIZING/THICKENING/GELLING AGENT ON FRUIT RIPPLES

Viscosity of continuous phase is primarily affected by the presence and level of thickening substances, especially polysaccharide hydrocolloids/gums and starches which are used to produce fruit preparations. Thus hydrocolloids and starches are the most essential food additives in fruit ripples and toppings. For optimal function of the gelling agent or thickening agent, it is necessary to know thoroughly about the requirements for proper gel formation. Failing to this requirement may frequently contribute to products of undesirable consistency. An ideal gelling agent should not interfere with the odor, flavor, or taste of the fruit ripple.[18] The water-holding capacity, flow behavior as well as melting behavior of fruit ripple depends on the amount and type of stabilizing ingredients added.[10,11]

2.2.2 HYDROCOLLOIDS/GUMS AND THEIR COMBINATIONS

The flow properties and rheological behavior of hydrocolloids play a significant role in the food industry as it governs the product development, designing of the process equipment, and so forth. In fruit preparations and artificially sweetened foods, hydrocolloids/gums provide good rheological properties by imparting viscosity or thickening, which governs the end quality of the food product. Thickening depends on the type and concentration of gum in the food product.[20] The gums/hydrocolloids, have functional properties in stabilizing the insoluble particles, thickening, improving the consistency, and are more sensitive to changes in the solid contents.[32] The gum shows shear thinning behavior even at low shear rate (1–50 s^{-1}).

Polysaccharide hydrocolloids (such as: pectins, xanthan, guar gum, tragacanth, and sodium alginate) are used generally as hydrocolloids, to increase the rheological properties of fruit ripple.[30] Other than hydrocolloids, different botanical origin starch and their modified version are also used for thickening or gelling purpose in fruit ripples.

Pectin is being used as a gelling agent in fruit preparation, but unfortunately, pectin network alone cannot withstand the mechanical stress and gets damaged, leading to syneresis affecting the baking quality of baked food incorporated with fruit filling. This problem can be solved by integrating pectin with other thickening agents. Thus, the interest has grown for formulating blend of hydrocolloids with novel functional properties.

Gellan gum forms a transparent gel in the presence of multivalent cations, which is resistant to heat and acid. Inulin addition to fruit ripple helps to prevent syneresis in fruit fillings, due to its good water-holding, gelling, and thickening capacity and it also acts as a bulking agent, imparting rheological properties to the fruit fillings. Similar results were obtained by Evageliou et al.[13] while studying thermal-stable fruit fillings. Cropotova et al.[10] stated that addition of inulin and pectin to the fruit fillings' composition, in order to provide heat-stability, pumpability, absence of syneresis and separation of fruit, also a proper degree of "*restructuring after shear, may increase the total antioxidant activity. Linear models showed that the pertinent of the addition of pectin and gellan gum to fillings prevents syneresis, increases water-holding capacity particularly after freezing.*[10,11]"

Xanthan gum exhibits pseudoplasticity and thixotropy with high viscosity at low concentration.[32] Low xanthan containing fruit preparation samples show reduction in consistency during storage.[25] Xanthan gum are more heat stable and can be added with the native starch or modified starch for heat stable fruit preparation.[8] Nalawade et al.[27,28] studied the effect of xanthan gum, guar gum, and pectin on different combinations for the development of mango, strawberry, and pineapple fruit ripple and reported that the addition of xanthan gum helps to provide optimum rheological characteristics to the ripple and avoid syneresis. Similar results were also obtained by Oczad[30] Locust bean gum (LBG) is used as a thickening agent to give light texture, which can recover its structure even after subjecting to shearing. LBG helps to reduce syneresis and improve rheological characteristics more effectively as compared to xanthan gum. The ''hairy'' regions of LBG (branching units clustered in blocks) are responsible for the network's dispersibility via hydrogen bonding with water molecules thereby inhibiting syneresis.[25]

Due to low viscous characteristics, gum arabic is compatible with most of the other gums, which can provide smooth flow in combination with other gums like xanthan, gelatin, agar, guar gum, and modified starches in confectionary products. The gum is of beneficial use when a thin, pourable consistency is desired.[20] Wei et al.[36] studied the effect of addition of gums, in the fruit ripple/filling, on viscosity and found that the guar gum, LBG, and CMC increases the consistency and flow indices in modified Herschel–Bulkley equation while those decreases with the addition of xanthan gum and κ-carrageenan. Thus the apparent viscosity of fruit fillings/ripples varies with the added gum type and its amount. Onweluzo et al.[31] showed that Detarium microcarpum polysaccharide increases viscosity and had a stabilizing effect in fruit preparation. Hydrocolloids and their combinations for thickening and texture modification are one of the most important ingredients to be used

in fruit preparation, gravies, pet foods, various beverages, and dairy products as their rheological and functional properties are complimentary.[20]

2.2.3 NATIVE STARCH AND MODIFIED STARCH

Starches are polysaccharide consisting of linear (amylose) and branched (amylopectin) alpha-glucans. Starches from different botanical sources (such as rice, wheat, corn, potato, and wheat) are used in the food industry for their thickening and gelling ability in different fruit preparations.[21,32]

However, native starches are not frequently used in food,[34] as it is unable to give rheological stability to fruit ripple and is also low resistance to mechanical, thermal, and chemical agents, leading to lower end-product quality.[2,3] Moreover they also undergo retrogradation and syneresis, which limits its usage as a thickening agent. The solution for these problems brings the production of specialty starches.[34] Chemical, physical, and enzymatic modification of native starch is one of the best ways to overcome the inabilities of native starch. The resulting modified starch can have different functional properties and hence can be used as gelling, thickening, texturing, bulking, stabilizing, and filling agents in food production.[30,32] Etherification of starches can give stability against retrogradation and cross-linking can give stability against acid, thermal and mechanical degradation.[2,3]

Cross-linked stabilized starches (phosphates, acetate, and hydroxypropyl ether starches derived from tapioca, potato, and waxy maize) are used as stabilizers/thickeners in fruit pie fillings, fruit preparation, toppings which can maintain viscosity even at high acidic preparation and high solid concentration. Hydroxypropyl starches are generally cross-linked to obtain desired freeze thaw stability, resistance to the high temperatures, low pH and shear degradation and texture loss encountered during food processing.[34] Hydrolyzed heat treated ungelatinized starch are good for preventing syneresis in vegetable or fruit-based products. Such modified starches are relatively easy to cook with high solids which help to provide a short texture and comparatively low viscosity during processing of fruit ripples. Pulpy texture resembling fruit pulp rheology can be obtained using highly cross-linked tapioca or corn pregels.[34]

Pregelatinized starches are also used as a thickening agent for fruit filling, which are useful to maintain rheology of filling without application of heat which helps to avoid the loss of the fresh flavor of the fruits.[34] Xanthan gum-modified starches have heat and shear stability. Waxy rice starch with 98% amylopectin gives a creamy gel texture and natural heat and freeze-stability.

Waxy starch with high amylose starches can produce gels of good strength and stability to syneresis.[34] Sago starch can be used in fruit preparations as thickening or gelling agent. There is direct relationship between concentration of sago starch and their ability to form a more rigid gel structure.[21] The problems of native starch can be overcome by using mixed system of native starch and hydrocolloids, which could act with starches through macromolecular interactions.[2,3]

2.2.4 MIXED SYSTEM OF HYDROCOLLOIDS AND STARCHES AS THICKENING/GELLING AGENT

Use of mixed system (hydrocolloids and starch) in fruit preparation leads sharp rise of viscosity due to contribution of both, hydrocolloids in continuous phase, and starches in dispersed phase.[3] Chemical modification of starch can be replaced by the utilization of its interactions with hydrocolloids that may stabilize its properties.[32] Agudelo et al.[2,3] studied the effect of addition of low methoxyl pectin (LMP) with tapioca starch on rheology of fruit fillings. Khondkar et al.[22] studied the effect of LMP on the dynamic rheological properties of cross-linked and uncross-linked waxy corn starch. Both the group of scientist found that G′ and G″ increased when pectin was added, due to cross-linking between the starch and the pectin, showing a sizeable increase in viscoelastic behavior and a significant drop in tan α (closer to 0), reflecting increased viscoelasticity. Further Agudelo et al.[3] stated that the values of G′ and G″ for modified tapioca starch are slightly higher than those of native tapioca starch, which reflects the stronger gel structure in the modified starch.

Sikora et al.[35] used starch (potato, tapioca, oat, and corn separately) and xanthan gum in a proportion of 9:1 and suggested that initially starch and gum gel solution forms two separate phases. These phases then combine into one phase, leading to increased viscosity. After combination they lead to separation of phase again, causing breakdown of structure which in turn results in reduction of viscosity. Wei et al.[36] obtained same results with hydrocolloids (guar gum, LBG, carboxymethyl cellulose, xanthan gum, κ-carrageenan separately) and waxy corn starch addition in fruit fillings. Agudelo et al.[3] studied the effect of addition of carrageenan or pectin to native tapioca starch in fruit filling. They concluded that the addition of carrageenan to native tapioca starch in fruit preparation leads to increased viscosity as compared to addition of pectin and tapioca starch. The results obtained by Pichler et al.,[32] while studying the effect of waxy maize starch and tapioca starch on

raspberry filling, showed that the waxy maize modified starch or guar gum additions had a greater impact on the consistency than the tapioca modified starch or gum karaya. Waxy maize starch gave better consistency and rheological properties to the fruit filling than tapioca starch alone. Galkowska et al.[15] studied the chemically modified potato starch-pectin-sucrose systems for fruit filling and found that the starch-pectin-sucrose gels exhibited better structuring ability.

2.2.5 EFFECT OF ADDED SUGAR AND ACID REGULATORS ON FRUIT RIPPLES

Sucrose is one of the major ingredients in fruit fillings. Corn syrup, inverted sugar, liquid glucose are also can be used as sweetening agents. The use of inverted sugar, corn syrup, liquid glucose prevents crystallization and helps to improve rheological properties. Fondant sugar can be added to the jelly base, fruit preparations for providing creaminess.[26]

Sugars plays important role other than sweetening in many food products containing starch;[38] they affect properties such as gelatinization, retrogradation, and staling. Many mechanisms are proposed to explain the interactions between sugars and starch to achieve the required properties and product stability: a) starch–sugar interaction by stabilizing junction zones and increasing the melting temperature,[38] b) the formation of starch–sugar inclusion complexes, c) increase in free volume resulting in a less plasticizing effect of the starch–sugar solvent.[32] In hydrocolloid mixture 55–70% sugar concentration stabilizes the junction zones within the gel network by a complex mixture of hydrogen bonds, hydrophobic and electrostatic interactions.[38]

Agudelo et al.[2,3] observed that the viscosity values and pasting temperature increase with the sugar concentration. Chantaro and Pongsawatmanit[7] also obtained same results along with increase in peak viscosity, cold peak viscosity, breakdown and setback values in mixtures of native tapioca starch and xanthan gum. Pongsawatmanit et al.[33] found while studying the effect of mixtures of native tapioca starch and xyloglucan on rheology of fruit preparation, that the final viscosity peak increased with the increase in the quantities of sugar.

Stabilizers, more specifically hydrocolloids, work to the best at their isoelectric point (pKa) value. At isoelectric pH, the net charges on the hydrocolloids get nullified and their structure acquires a steady conformation leading to firm structure of gel. The pH is more important for proper bonding

of gums and proper gel formation at just above pKa value. And thus acid regulators play most important role while processing and also during storage period, in gel formation and consequently viscosity and syneresis. Starches have negligible effect of fluctuation in pH.[2,3] While studying the effect of pectin in syneresis stated that the increase in pH toward the pKa value leads to increase in the calcium–chelate bonding site. This helps the Ca^{++} ion to make bonding with the pectin molecule very effectively. Further, increase in the pH, beyond pKa value causes loosening of gel structure, leading to syneresis and heat sensitive structure.[38] Similar findings were observed by Nalawade et al.[27,28] while working with mango, strawberry, and pineapple fruit ripple.

Hardness of mango jam increased with the pectin concentration and acidity. Hardness increased upto 60% sugar concentration but was found to decrease with further increase in sugar concentration at all pH and pectin levels.[6] Guerrero and Alzamora[16,17] studied the effect on rheological properties of fresh peach, mango, banana, and papaya purees, on the addition of glucose and adjusted pH. They found that, the rheological parameters were temperature and soluble solids content sensitive. The consistency coefficient increased with increasing soluble solids content (increasing added sugar) and decreasing temperature.

Lootens et al.[23] showed that the viscosity of fruit ripple containing pectin increased on addition of Ca^{++} ions at reduced temperature, as the Ca^{++} ions interact with pectin to form a firm gel. Agudelo et al.[2,3] observed that pH fluctuations between 4.2 and 9.2 had no significant effect on rheological characteristics of tapioca starch. In native rice starch and hydroxypropylated rice starch, addition of calcium ion causes reduction in viscosity.[19] These authors suggested that the interaction of the calcium and starches resulted in formation of chelates by means of intra- and intermolecular hydrogen bonds which created cross-links between the molecules or within the same molecule through the coordination of two anionic oxygen (ionized hydroxyl groups), which affected the starch behavior.[3]

2.2.6 EFFECT OF PROCESSING CONDITIONS ON FRUIT RIPPLES

Processing conditions predominantly affect the rheological properties of fruit ripples. Processing conditions includes temperature at which the fruit ripple is heated, time for which the heat treatments is employed and shear force at which the ripple is pumped during its application. These conditions

should be optimal. Lowering in the values of time and temperature may not produce the fruit ripple of desired quality due to underutilized capacity of stabilizer. Even, exceeding the optimal conditions of time and temperature during processing the fruit ripple may cause damage to the gel structure. Excess shear force is also one of the reasons for damaging the gel network causing decrease in rheological properties.[27,28]

Temperature is one of the important processing parameters, which affects the rheological characteristics of hydrocolloids and fruit preparation. During fruit ripple preparations, two heating steps must be considered: heating during the fruit filling preparation (an intermediate step in industrial manufacture) and during baking of the pastries. Heating affects the quality of the native starch-based fruit fillings, due to gelatinization and degradation of the starch upon heating at low pH values.[2] Arrhenius relationship can be used to study the effect of the temperature on rheological characteristics at constant shear rate, while an exponential or power relationship can be used to study the effect of concentration on apparent viscosity of hydrocolloids and fruit ripple.[6,20]

Dependence of the flow behavior of fluid foods on temperature can be described by following Arrhenius relationship:[6]

$$K = A_K[\exp(E_K/RT)] \quad (1)$$

Where, K is consistency coefficient (Pa.s^n), A_K is frequency factor (Pa.s^n), E_K represents activation energy (kJ/mol), R is gas law constant (R = 8.314 J/mol K), and T is absolute temperature (K).

From a practical standpoint, it will be more relevant to describe the effects of temperature and gum concentration on flow characteristics by a combined model. Jasim et al.[20] used the combined effect of temperature and concentration on consistency index/apparent viscosity to describe the flow behavior of hydrocolloids. The magnitude of yield τ_0, decreased with increase in temperature while gum concentration increased the same amount.[32] Espinosa et al.,[12] studied the rheological properties of fruit purees after heat treatment with the help of Herschel–Bulkley model and stated that the heat treatment affected the cell wall structure resulting in the degradation and solubilization of pectins leading to decrease in viscosity and changes in the rigidity of particles of the puree and the serum.[4,12]

Effect of temperature on rheological properties of fruit ripple contemplates thermal instability of fruit fillings in baked products while baking and syneresis during the storage. Heating of ripples while baking, induces loss of structural stability due to changes in the conformational structure of the gel

network, which in turn engenders syneresis. Syneresis also may be caused during thawing, when ripple impinge with the dough, leading to poor dough quality, less acceptance and reduced quality of final product. These all are rheological defects caused due to improper capability of stabilizers to withstand the heat fluctuation. Hence during selecting stabilizers for preparation of fruit ripples to be used in bakery products, it is of prime importance to look for heat stability and syneresis free fruit ripple.[10,11]

Cropotova et al.[11] studied pectin and gellan gum as a gelling agent in the fruit filling for thermal stability in baked food and reported their usefulness in achieving thermal stability to fruit filling after baking. They also stated that the hydrocolloids can provide an excellent thermal stability in different food applications, including fruit fillings; by acting on the melting and freezing points of fruit fillings, leading to the stability to initial structure at high and low temperatures.[10,11] For bake stable fillings, the breaking strength of gel increases with increasing sugar content upto 70%, while higher content of sugar, destabilizes junction zones, leading to poorer bake-stable fruit fillings. Calcium alginate/alginate can give bake stability and reduce syneresis defects.

Pectin, just above the pKa value creates more potential calcium–chelate bonding sites leading to decreased syneresis. Young et al.[38] showed that alginate and pectin if used in combination can be beneficial in high soluble solids fruit fillings, with respect to bake stability, thixotropy and pumpability. The reason behind this is antagonistic competition between the alginate and pectin for the available calcium.[38] Tapioca starch with xanthan gum is more heat stable and the firm gel gives higher value of G′ and G″ than tapioca starch alone. Reheating the fruit preparation up to 90 °C and cooling to 10 °C and holding for around 10 h can increase the starch gum binding which can lead to formation of firm gel.[8] Hydrocolloids like carboxymethyl cellulose and pectin are usually used to produce the consistency required in jams and also to overcome syneresis problems due to temperature fluctuations.[21]

2.2.7 RHEOLOGICAL MODEL FITTING FOR FRUIT RIPPLES

Generally, hydrocolloids and starch depict non-Newtonian behavior and they impart non-Newtonian character to the emulsion even, when the amount of the dispersed phase is low. Fruit ripples/sauce/toppings, when added with hydrocolloids, act as non-Newtonian pseudoplastic fluid. Starch blends are usually pseudoplastic in nature when granules are significantly swollen,

which leads to their deformation under shear force.[25,27,28] The stability of gel structure under shear stress with time can be analyzed with the help of different time dependency models.[6]

Oscillatory tests can be used for determination of the viscoelastic properties such as G′ (storage modulus) and G″ (loss modulus) also model fitting with frequency variation between 0.1 and 10 Hz. Tension and viscosity curves by submitting controlled shear rate with rotational test allows characterization of fruit fillings.[26] In viscoelastic material, the storage and loss modulus measures the stored energy (elastic portion) and the energy dissipated as heat (viscous portion), respectively. The fruit ripples are viscoelastic in nature as they have both, that is elastic and viscous properties of fluid. In the oscillatory tests of fruit ripples, stress decreases with time starting at some high value and decreasing to zero. Also the strain rate decreases with time in the initial zone of the graph, until finally reaching a steady state. Further in the recovery zone (next half period of the graph), the viscoelastic fluid eventually start reaching an equilibrium at some small total strain relative to the strain at unloading.

The following Herschel–Bulkley model can be fitted adequately with minimum standard errors:[6,16,17,21]

$$\tau = \tau_o + K\gamma^n \quad (2)$$

Where, τ is the shear stress (Pa), τ_o is the yield stress, γ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^n$), and n is the flow behavior index (dimensionless), which signifies the extent of deviation from the Newtonian behavior.[6,20]

Yield stress can be defined as a point when the curve of shear stress versus shear rate, starts showing departure of shear rate from zero. In simple terms, this condition is an indication of shear stress at which the material start flowing, that corresponding shear stress is considered as the yield stress.[6]

The apparent viscosity and values of G′ and G″ of fruit preparations increases with increase in starch or hydrocolloids concentration and decrease with temperature Javanmard et al.[21] According to Ostwald–de Waele constants, a gum addition results in consistency (k) reduction and flow index (n) increases, resulting in less pseudoplastic samples, which seems that the resistance to shearing forces is increased as macromolecules' entanglements increased due to a higher polymer concentration in the emulsion.[25] Mandala et al.[25] stated that fruit sauce with xanthan gum and LBG are pseudoplastic in nature. Xanthan gum containing sauce is more pseudoplastic than LBG.

KEYWORDS

- **acidity regulators**
- **fruit filling**
- **fruit ripple**
- **Herschel–Bulkley model**
- **hydrocolloids**
- **loss modulus**
- **Newtonian pseudoplastic fluid**
- **oscillatory tests**
- **rheological properties**
- **starches**
- **storage modulus**
- **sweetening agents**
- **tapioca starch**
- **xanthan gum**

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CHAPTER 3

ROLE OF EMULSIFYING SALTS IN CHEESE PRODUCTS

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CONTENTS

ABSTRACT

In order to prepare processed cheese, cheese spread, or cheese analogs, emulsifying salts (ES); one important food additive is indispensable. Such ES modifies the dominant protein network in cheese system to provide several functional properties viz., body and texture, melting quality, flowability, fat leakage, stretch, shred quality, etc. The two major types of ES used during manufacturing of processed cheese are sodium salts of citrates and sodium salt of phosphates, including polyphosphates. The type and rate of incorporation of an ES varies as per the type of cheese product, the maturity of cheese base, or the type of protein concentrate used in cheese analog. Judicious blend of ES can exert greater impact on cheese properties than is exerted by individual ones. Several commercial food ingredient manufacturers take advantage of this synergy among ES to produce proprietary blend of ES that gives miraculous effect in cheese systems. In case of cheese analogs, the ES plays a still greater role since dried protein ingredients are used hydrate for obtaining the desired cheese quality.

3.1 INTRODUCTION

Dairy products like processed cheese and processed cheese spreads are manufactured by blending different types of cheese of varying ages, in presence of food additives (white butter, emulsifying salt (ES), NaCl, color, preservatives, water, etc.) followed by heating, cooling, and packaging. Cheese analogs are substitute of natural cheese that are made using processed cheese manufacturing technique, but utilizing raw materials like rennet casein or acid casein or Na-caseinate or vegetable proteins as protein source, and vegetable fat, or even milk fat as the fat source, along with other ingredients (viz., cheese flavor, NaCl, acid, color, preservative, etc.).

Consistency of processed cheese is affected by its composition, microstructure, the physicochemical state of its constituents, and its macrostructure. The factors, which govern the consistency of the processed cheese can be affected by many factors, such as maturity of natural cheese, pH of cheese melt, type and concentration of ES, processing temperature, speed of agitation, duration of heating, rate of cooling, temperature of storage, dry matter content, fat content, presence and concentration of ions (especially calcium, sodium and potassium), type and concentration of lactose or other sugars, using of emulsifiers (e.g., monoacylglycerides), etc.[12, 15, 37]

Each food additive has its own contribution in cheese systems. Among various food additives, ES play a major role in the manufacture of processed cheese, processed cheese spread, and cheese analogs. Similar to the proprietary blend of stabilizer-emulsifiers for the ice cream industry; several manufacturers are taking advantage of the proprietary blend of ES that are claimed to give excellent functionality to the processed cheese makers.

Understanding of the role of ES in such products can help the cheese maker to improve the product quality and may enable the food processors to "tailor-make" the cheese as per the whims and wishes of the retailers and consumers.

3.2 EMULSIFYING SALTS

ES, also referred to as "melting salts" or "chelating salts" are Na, K or Casalts of citrate, or phosphates (short or long chained), preferentially used as an ingredient in the manufacture of processed and imitation cheeses. Such additive helps in forming the structure of food and exerts positive influence on the melting properties by aiding emulsification of milk/vegetable fat in the protein matrix. The most commonly used ES are phosphates and citrates. On a mole for mole basis, phosphates have a higher calcium chelating ability than citrates.[24] The chelating ability of phosphates increases with increasing P_2O_5 content, poly- > pyro- > orthophosphates. ES provide uniform structure during the melting of process cheese and also affect the physical, chemical, and microbiological quality of resultant product.[11,34,67]

3.2.1 PROCESSED CHEESE, CHEESE SPREADS AND CHEESE ANALOGS

3.2.1.1 PROCESSED CHEESE AND SPREADS

The ripened cheeses of various ages are mixed in certain proportion and processed in presence of ES to yield "processed cheese/processed cheese spread." The processed cheese spread is formulated to have higher milk fat and moisture content so that it has the property to "spread" during food applications.[61] Processed cheese or cheese spread is manufactured by blending shredded natural cheeses of different types and degrees of maturity with ES, water, salt, milk fat, and heating the blend (80–85 °C/4–8 min) with constant agitation until a homogeneous mass is obtained. The product is subsequently stored at 5–10 ˚C after packaging and cooling (Fig. 3.1).[7] The legal requirements for processed cheese and cheese spread in India are shown in Table 3.1.

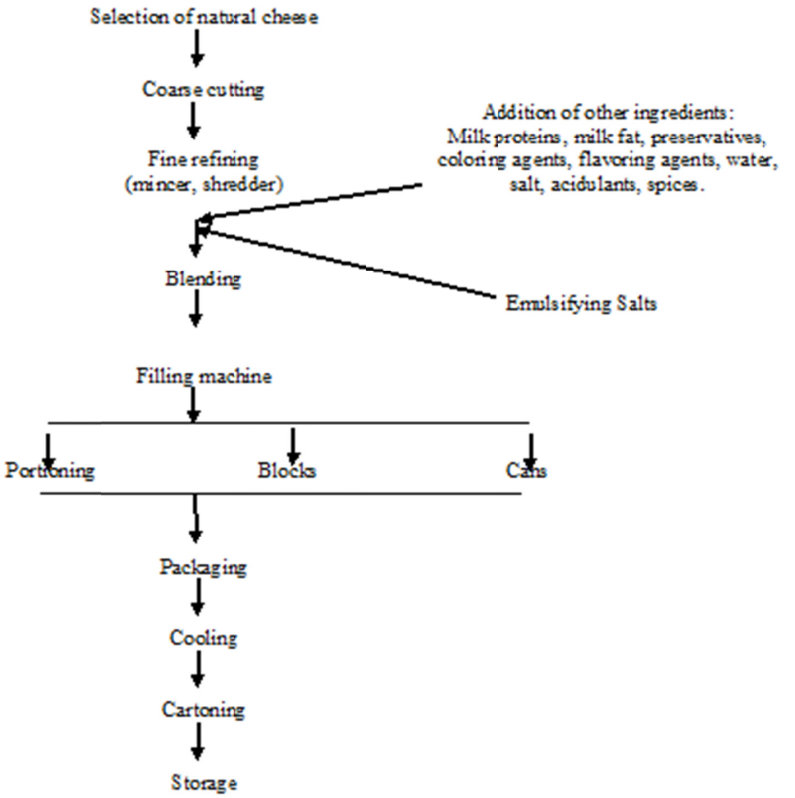

FIGURE 3.1 Schematic flow chart of the basic steps involved in process cheese manufacture.

TABLE 3.1 Standards for Process Cheese, Process Cheese Spread, and Pasteurized Process Cheese.

Particulars	Processed cheese30	Processed cheese spread30	Pasteurized process cheese
Moisture (%), max.	47.0	60.0	43.0
Fat on dry matter (%), min.	40.0	40.0	47.0
Lactose (%), max.	5.0	5.0	–
Na, K, Ca-salts of citric acid, orthophosphoric-acid, polyphosphoricacids (ppm), max.	40,000	40,000	30,000
Sorbic acid including its Na, K, Ca-salt (ppm), max.	3000	3000	2000
Nisin (ppm), max.	12.5	12.5	–
Lecithin (ppm), max	–	–	200

3.2.1.2 CHEESE ANALOGS

Cheese analogs also referred to as imitation cheeses are the products made out of dairy, partial dairy or even non-dairy ingredients that tend to resemble a particular cheese variety. Examples of analog cheeses are mozzarella cheese, cream cheese, process cheese, etc. Cheese analogs are prepared by blending dry ingredients (e.g., rennet/acid casein or caseinates, common salt, starch, cheese flavor) in the aqueous solution of ES followed by heat processing (~80–82 ˚C/3–5 min) with simultaneous incorporation of vegetable or dairy fat. An appropriate acid consisting of one or any mixture of two or more of vinegar, lactic acid, citric acid, acetic acid, and phosphoric acid (in solution form) is used to adjust the pH but not below 5.3 of cheese analog (Fig. 3.2). Further, processing is carried out in a manner similar to processed cheese.[18,58] Imitation cheese products are mainly used in the catering industry and in the processing sector, for example, in preparation of pizza and prepared dishes.[48]

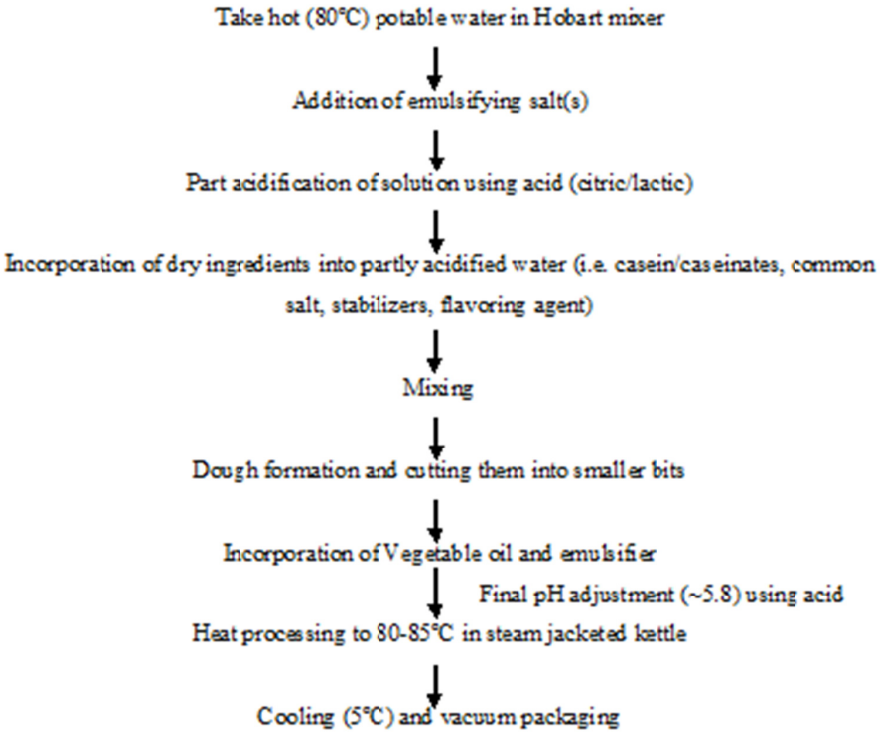

FIGURE 3.2 Schematic flow chart of the basic steps involved in cheese analog manufacture.

3.3 NEED FOR FAT EMULSIFICATION IN CHEESE

All cheese products are essentially "oil-in-water" emulsions. Natural cheese is a nearly perfect emulsion stabilized by natural surfactants, the cheese proteins. The principal caseins in cheese (αs_1-, αs_2-, β) have non-polar, lipophilic C-terminal segments, while the N-terminal regions containing calcium phosphate are hydrophilic. This structure allows the casein molecules to function as an emulsifier.[59] During cooking of processed cheese, the proteins from the cheese network are dispersed which then re-associate to form a protein network structure as cooking continues, resulting increase in viscosity. The change in viscosity of processed cheese gives rise to "creaming reaction", and well-hydrated and dissociated protein particles are said to be "under-creamed;" while protein particles that are optimally linked together as a strong gel network is a well-"creamed" and if too closely compacted, the processed cheese is called as an "over-creamed" cheese.[43] ES are added to promote the peptization of the casein matrix, which allows caseins to act as emulsifiers for milk fat. Addition of ES hydrates the casein, calcium ions are chelated and pH level rises which in return promotes peptization of the casein matrix. Processed cheese properties like meltability, free oil formation, and globule size decreases, whereas firmness increases with the increase in degree of emulsification of fat.[22] In case of processed cheese slices rheological properties, meltabilities, and microstructures are altered after addition of maize starch. Phase behavior of the starch in the protein matrix alters the properties of cheese slices. When starch (gelatinized or ungelatinized) is present as small particles and well dispersed in the protein matrix, it can affect the meltability of cheese. When starch (high level of gelatinization) forms a stranded network, which separates from the protein matrix to form a continuous phase of starch, the meltability of cheese decreases substantially.[7] However, in absence of ES, heating of natural cheese blend (i.e., of different maturity) above 65 °C can result in separation of oil and water phases. To incorporate the fat source (butter/vegetable fat for process cheese and cheese analog, respectively) in the proteinaceous cheese matrix, ES helps in this regard. Emulsification of fat in cheese is affected by factors like amount of calcium in the $CaPO_4$ end, pH, age of cheese, and temperature during cheese processing.[14]

3.4 CLASSIFICATION OF EMULSIFYING SALTS

ES are important ingredients in cheese making because heat treatment of natural cheese causes "oiling off," which is separation of fat from cheese

mass. The ES used for processed cheese systems are mainly classified into two types: citrates and phosphates.[49]

3.4.1 CITRATES

These are salts of citric acid, for example, tri-sodium citrate (TSC), which are preferred in-block type cheese and sliceable cheeses. Citrates are highly soluble with fairly good protein dissolving power. They contribute to long, elastic structure to processed cheese; the structure of cheese remains firm and heavy. Few disadvantages of citrates are lack of creaming, lack of bacteriostatic action and risk of sandiness, and mottling in cheese.

3.4.2 PHOSPHATES

They are salts of phosphoric acid. There are two types of phosphates: Monophosphates (orthophosphates: NaH_2PO_4, Na_2HPO_4, and Na_3PO_4) and condensed phosphates ($Na_6P_4O_{13}$ $(NaPO_3)_n$).

3.4.2.1 MONOPHOSPHATES/ORTHOPHOSPHATES

These include mono-sodium, di-sodium, and tri-sodium orthophosphates (TSP). Monophosphates are water-soluble and have excellent buffering properties, but do not exert any creaming effect. Mono-sodium and TSP are most commonly used for pH adjustment.

3.4.2.2 CONDENSED PHOSPHATES

These are classified into chain forming polyphosphates, ring-forming metaphosphates and cross-linked ultraphosphates. The chain forming polyphosphates are classified as short- and long-chain polyphosphates. Polyphosphates have strong creaming action and exert bacteriostatic property. Their buffering capacity depends on their chain length.

3.4.2.2.1 Short Chain Polyphosphates

These are mainly the triphosphates and tripolyphosphates with pH value of 1.0% solution ranging from 2.7 to 10.2. The commonly used salts are mono-sodium, di-sodium, tri-sodium, and tetra-sodium diphosphates.

3.4.2.2.2 Long Chain Polyphosphates

These have an average degree of condensation ranging from 4 to 25. Graham's salt (i.e., sodium hexametaphosphate – SHMP) with a degree of condensation of 10–25 has outstanding ion-exchange ability. The disadvantage is their proneness to partial hydrolysis during processing. The pH of commonly used ES (as 1.0% solution) is depicted in Table 3.2.

TABLE 3.2 Commonly Used ES and Their pH.[49]

Emulsifying salts	**pH value (1.0% solution)**
Citrates	
Monosodium citrate	3.8
Disodium citrate	5.1
Trisodium citrate	8.2
Orthophosphates	
Monosodium monophosphate	4.5
Disodium monophosphate (DSP)	9.0
Trisodium monophosphate (TSP)	11.5
Short chain phosphates	
Monosodium diphosphate	2.7
Disodium diphosphate	4.2
Trisodium diphosphate	7.0

3.5 ROLE OF EMULSIFYING SALTS IN CHEESE MAKING

Melting of cheese during its application, ES are used in order to modify the cheese proteins in natural cheese, which can emulsify the fat during processing and also to develop certain characteristics in processed cheeses like uniform body. The mechanism of action of ES in any cheese system involves:

a. Removal of calcium from the protein system.
b. Peptizing, solubilizing, and dispersing the proteins.
c. Hydrating and swelling of the proteins.
d. Emulsifying the fat and stabilizing the emulsion.
e. Controlling pH.
f. Forming an appropriate structure of the product during or after cooling.[11,16,17]

The monovalent Na^{+} and bivalent Ca^{++} ions are antagonistic with regard to their action on proteins. The calcium bound to casein fragments depresses their water solubility. ES removes calcium by exchanging it for sodium or by binding to it.[29,33] The properties of ES are collated in Table 3.3. The functional behavior of cheese products are strongly influenced by the type of ES and its physico–chemical properties including its ability to bind calcium, casein dispersion during cooking, and creation of cross-links with casein during cooling.[21]

TABLE 3.3 Properties of ES Applicable to Processed Cheese Products.

Emulsifying salts	Ca sequestration	Buffering action	Para-casein hydration	Fat emulsification
Citrate and Orthophosphate	Low	High	Low	Low
Pyrophosphate	Medium	Medium	Very high	Very high
Polyphosphate	High to very high	Low to very low	High to low	Very high to low

3.5.1 TYPES OF EMULSIFYING SALTS

The most commonly used ES for process cheese manufacture are TSC and DSP. TSC is preferred ES for slice-on-slice process cheese varieties, whereas DSP (or appropriate combinations of di- and trisodium phosphates) is used in loaf-type process cheese and process cheese spreads. Sometimes low levels of SHMP are also used along with these ES salts.[49] The quality of emulsifying agent added to processed cheese blend depends on the type and age of cheese used in the blend (proportion of water and calcium), but is also determined by the final product group (i.e., block, spread, slices, etc.). The ES that help in structure building (not creaming) include high molecular polyphosphate JohaC, JohaSE, JohaS7, JohaPZ and citrate, which leads to firm, slicing processed cheese. ES that lead to creaming, for example, lower

and medium molecular polyphosphate, JohaS9, JohaS9 special, JohaS10, JohaS90 are suitable for spreadable processed cheese.[68]

Kairyukshtene[39] observed that when 3% solution each of orthophosphate, pyrophosphate, tripolyphosphate, citrate, and SHMP was used to manufacture processed cheese. Fine structure and fat dispersion was best obtained when using citrate, tripolyphosphate, and orthophosphate in processed fresh or moderately ripened cheese. Linear polyphosphates (with ≥ 4 phosphates) are obtained from very pure orthophosphates (monophosphates) by high-temperature condensation. They are hydrolyzed in an aqueous solution; this occurs during melting and continues during storage of processed cheese. The hydrolysis quickly leads first to triphosphates and diphosphates (pyrophosphates) then more slowly to orthophosphates, which are excellent pH buffers. A part of added polyphosphates gets hydrolyzed during the melting process; rest is hydrolyzed after 7–10 week of storage.[16] Commercial mixtures of ES often contain short-chain oligophosphates with the required good balance of calcium binding and buffering properties. Long-chain polyphosphates are added to improve calcium binding. A combination of Joha S9S + Joha NO (1:1) ES at 3.0% w/w is suitable to produce processed cheese spread containing WPC; such incorporation can affect the pH, free oil, meltability, and sensory properties of the product.[8]

3.5.2 RATE OF ADDITION OF AN EMULSIFYING SALT

The amount of ES that can be added to the cheese base are regulated by many countries and usually do not exceed 3.0–4.0%. However, a cheese maker would tend to use the minimum quantity that gives the desired effect in cheese system. Increased levels of polyphosphate in ES mixtures (with pyrophosphate or orthophosphate) results in a firm processed cheese spread based on Ras cheese, whereas increased levels of pyrophosphate can produce softer cheeses and the reason is attributed to the buffering capacity.[10] The degree of casein dissociation, pH, and hardness of processed cheese increased as the concentration of ES was raised. The processed cheese prepared with TSC was whiter than those prepared with other ES.[19] Hardness of non-fat Pasta filata cheese will decrease, while the meltability can increase with increasing concentrations of TSC from 1.0 to 5.0%. The effect can be attributed to a decrease in the number of colloidal calcium phosphate cross-links and an increase in electrostatic repulsion in the cheese system. Addition of 1.0% TSC increased the stretchability of cheese by partially loosening the protein matrix.[50] In addition, the focus of investigation has been largely on

processed cheese while little work has been done on imitation cheese, where the interaction of ES with casein is still not clearly understood. The progressive increase in hardness and decrease in flowability values at reduced ES levels may be related to the observed decrease in fat globule sizes. Cavalier-Salou and Cheftel[17] reported that at decreased ES, hardness of imitation cheese increased, and meltability decreased due to increased manufacturing times. Shirashoji et al.[60] also reported that high firmness and low-melting values in process cheese were associated with small fat globule sizes.

Decreasing the concentration of ES in processed cheese spread can led to increased free oil formation. The concentration of the ES also affects the sensory properties of the cheese product.[4] Meltability of processed cheese; made from ultra-filtered (UF) cheese base by utilizing cheddar cheese: UF cheese base in 70:30 proportion, increased with increase in ES. Park[53] reported that gumminess, brittleness, adhesiveness, cohesiveness, and hardness of processed cheese with 3.1–3.4% ES were higher than for other samples made using other levels of ES. Hardness, elasticity and adhesiveness of processed cheese with 4.0 or 4.3% ES were similar to those of commercial processed cheese; cheese containing 4.3%.

3.5.3 COMBINING EMULSIFYING SALTS

Single ES is not capable to provide all of the desirable characteristics to the processed cheese or cheese analogs. Random combinations of citrates and phosphates are also ineffective and hence right proportion of individual ES should be calculated and adopted. TSC and DSP when used in 1:2 ratios can yield an excellent quality processed cheese with desired firmness, sliceability, and smoothness. Such combination will help to strike a balance between the water absorption properties of diphosphate and the protein solubilizing property of citrates.[55]

The proprietary blends of ES available in the market include: JOHA HBS, JOHA S9, JOHA® – SOLVA®, SELF 690, SELF® SR, CREMOSAL, CHEESEFOS, and so on. In processed cheese spread, use of binary mixtures of polyphosphate combined with monophosphate, diphosphate, or triphosphate can led to a gradual increase in the hardness of product with increasing proportion of polyphosphates. In binary mixtures of monophosphate combined with diphosphate or triphosphate, a rapid increase in the hardness of the product can be observed when the proportion of di/triphosphate in the mixture is above 60.0%.[66]

Processed cheese spread based on Ras cheese when prepared using 2.5% of ES mixtures comprising of sodium pyrophosphate (PYR) + sodium polyphosphate (POL); PYR + POL + sodium tripolyphosphate (STPP); and PYR + POL + sodium orthophosphate + STPP, the pH values tended to decrease with increasing POL ratio. Soluble nitrogen content and peptization increased with increasing PYR content. Use of ES mixtures; PYR + POL, PYR + POL + STPP, PYR + POL + sodium orthophosphate + STPP in proportion of 70:30, 60:30:10, and 50:20:20:10, respectively, is recommended for preparation of spreadable processed cheese.[2,3] Shiny and whiter block type processed cheese from Ras cheese can be prepared using salt mixtures of sodium diphosphate, sodium polyphosphate, and STPP (30:40:30) and sodium polyphosphate, sodium citrate, sodium orthophosphate, and sodium diphosphate (50:20:20:10).[9,66] TSC alone and its combination with DSP can result in superior quality of processed cheese spread as compared to those made using TSC + TSP.[23]

3.6 IMPACT OF EMULSIFYING SALT ON CHEESE PROPERTIES

Instrumentation provides greater insight into cheese texture and structure. Instruments usually used for assessing the textural, structural, and rheological properties include transmission and scanning electron microscopy (TEM and SEM), confocal laser scanning microscopy (CLSM), dynamic oscillatory rheometry, and atomic force microscopy. Other more indirect methodologies have provided important information about cheese functionality, despite requiring less expensive equipment, such as measurement of free oil formation, the state of water (bulk, or bound to casein), color determination, and large-scale deformation texture profile analysis.[22] Since, inclusion of an ES has influence on milk proteins and hence its interaction with milk fat in cheese system, variation in the type and/or amount of ES is bound to affect the cheese properties. The impact exerted by use of ES in cheese system is discussed in the following sections.

3.6.1 IMPACT ON APPEARANCE

The whiteness of cheese spreads made with different types of ES was variable. Increasing the concentration of ES and incorporation of WPC both improved the whiteness of the processed cheese spreads based on Ras and Quark cheeses.[5]

3.6.2 IMPACT ON FAT GLOBULE SIZE

Process cheese made using 0.25% TSC had poorly emulsified fat globules, whereas use of 2.75% TSC led to desired fat emulsification in cheese.[60] Processed cheese made with SHMP had larger fat particle size (4.68 μm) than cheeses made with the other ES (2.71–3.30 μm).[19] ES such as tetra-Na-pyrophosphate (TSPP), Na-polyphosphate (SPP), and Na-tripolyphosphate (STPP) were tried out in various proportions in the preparation of processed Ras cheese and adopting the ratio (TSPP: SPP: STPP; 30:40:30 or 30:30:40) can give desired fat globule size distribution in cheese.[9]

3.6.3 IMPACT ON THE TEXTURAL AND FUNCTIONAL PROPERTIES OF CHEESE

During processing, the natural cheese, consisting mainly of insoluble calcium paracaseinate and fat globules are finely dispersed, homogenized, and converted into a gel, in which fat is emulsified. By chelating the calcium from the protein structure, the ES contribute to the dispersion of the proteins and enhance their emulsifying properties. Some of the properties exerted by ES on processed cheese or cheese analogs are shown in Table 3.4.

TABLE 3.4 Effects of ES on the Quality of Processed Cheese and Cheese Analogs.

Emulsifying salt	Ref.	Rate (%)	Effect of raising emulsifying salt concentration	pH change
Disodium phosphate	Ref.[5]	1.0–4.0	An increase in the firmness of processed cheese	5.6–5.8
Mono sodium citrate	Ref. [23]	1.6–2.6	Mealy, acidic, and crumbly with poor emulsification of fat; non-meltable, non-sliceable processed cheese	5.0–4.7
Na-hexa meta phosphate	Ref.[11]	0.25–2.75	The hardness of fat-free process cheese spread increase while its meltability decreased	–
Tri sodium citrate	Ref.[42]	1.0–3.0	Reduction firmness of processed cheese analog	–
Tri sodium citrate	Ref.[60]	0.5–5.0	Hardness of non-fat processed cheese decreased up to 2% TSC, while melt tendency increased; > 2% usage gave opposite effect	–
Tri sodium citrate	Ref.[9]	0.25–2.75	Increased hardness, decreased melt-ability of processed cheese	5.8–5.9

Physicochemical properties of processed cheese are usually affected to a great extent by the pH level. Cavalier-Salou and Cheftel[17] reported that the influence of ES (at 3% wt/wt usage level) on pH of analog cheese increased in the following order: tetrasodium pyrophosphate > TSC > disodium phosphate > sodium phosphate. Thomas[63] reported that the pH of pasteurized process cheese should be ≥ 5.6 to have suitable textural attributes. Cooking time also affects the textural properties and size of fat droplets of process cheese, prolonged cooking, and the types of ES causes changes in the viscosity of the molten cheese, which is the so-called "creaming" or creaming reaction.[67] Excessively long cooking time can result in the formation of a pudding like gel of molten cheese mass in the cooker (i.e., over creaming) or a crumbly, dry texture in the final product; both are considered defects.

Caseins re-associate or re-polymerize during cooling and form a new network between casein via hydrophobic, electrostatic interactions, hydrogen bonds, and some other types of interactions. It is possible that greater dispersion or exposure of groups on casein helps in the creation of a stiffer, less meltable matrix once cooled.[43] Lee et al.[42] also found that increasing moisture content resulted in the rheological behavior of processed cheese spreads changing from solid-like to liquid-like behavior. The effect of moisture content on the viscoelastic properties of processed cheese is to act as a plasticizer in the protein matrix, thereby making it less elastic.[29] When, the protein content is increased all the viscoelastic properties of processed cheese samples are increased, except loss tangent, which is decreased. Proteins are responsible for increased values of elastic and viscous moduli in processed cheese. Increasing concentration of caseins in the cheese matrix increases the intra- and inter-strand linkages, which are difficult to deform. During cooling, the role of proteins in the texture of the final product dominates over that of water and fat, resulting in products with increased viscoelastic properties, and a more solid-like behavior.[32]

Monophosphates have the lowest ability to exchange ions and hence when used in production of processed cheeses produces cheese with low hardness but with increasing number of phosphorus atoms in the molecule of phosphate used as an ES, higher values of hardness of processed cheeses are reached. Whilst triphosphates possess a strong ability to exchange ions in comparison with diphosphates and hence when used during manufacturing of processed cheese, the texture is too soft. On the other hand, triphosphates, and diphosphates have a lower ability to bind calcium into complexes than long-chain polyphosphates, which is in accordance with the results on the texture parameters of the processed cheeses with TSPP, PSTP, and POLY applied on their own.[28] Gupta et al.[35] reported that the firmness of process

Cheddar cheese increased with an increase in the concentration of TSC (1.2–2.1%; pH ~5.8) and predicted two mechanisms for the increased hardness and lower meltability. The two mechanisms were able to improve fat emulsification and greater dispersion of casein.

TSC and DSP can exhibit similar behavior and produce the softest, most adhesive, least elastic, and most liquid-like final processed cheese products, which can reduce apparent viscosity during processing. TSPP can produce harder, less adhesive, more elastic, and more solid-like processed cheese, with higher apparent viscosity than penta-STTP. SHMP can produce cheeses that can exhibit the least apparent viscosity during processing and the highest textural (except for adhesiveness and stringiness) and visco-elastic properties. With increasing number of phosphorus atoms in the molecule of phosphate, based ES, higher values of hardness of processed cheese was obtained.[24]

Cheeses made with DSP and SHMP usually have lower values for hardness while those prepared by using TSPP have highest values for hardness and gumminess, followed by cheese made with STPP, which might be associated with fat particle size.[19] According to Savello et al.[57] and Lee et al.,[42] the smaller the diameter of the globules, the greater the surface area, and the number of protein–protein bonds, making the network firmer.

Requeijao cremoso (cheese spread) cheese made with SHMP have lower melting index, whiter color, and higher values for hardness, gumminess, and adhesiveness compared to those of products made using TSC, STPP, and TSPP[20] Cheese made using TSPP recorded highest value for consistency index, which was attributed to the combined effect of high pH and homogeneous fat particle size distribution.[21]

The influence of phosphate type ES on firmness of cheese in increased order is: orthophosphate, polyphosphate, diphosphate, and triphosphate. The application of polyphosphates usually causes formation of firmer processed cheese and while those prepared with diphosphate and triphosphate are more rigid, due to their ability to support gelation in cheese matrix. Increasing the polyphosphate content (up to 50.0%) in the binary mixtures with orthophosphates or diphosphates will cause an increase in the firmness of processed cheese; above 50.0% level, it usually has an opposite effect.[56]

3.6.3.1 MELT, FLOW PROPERTIES AND SLICEABILITY OF CHEESE

Incorporation of DSP and TSC can produce fat-free processed cheese spread having properties very near to those of a full-fat cheese, with TSC

providing maximum meltability.[61] The variation in the TSC concentration (2.0–3.0%) will influence only the melt and flow characteristics, but not the unmelted textural properties of processed cheese.[31] Meltability of processed cheese will decrease significantly with increase in the ES concentration. Meltability of product in decreasing order for cheese containing ES: Trisodium pyrophosphate, DSP and TSC, and raising the rate of ES will result in a significant increase in hardness and significant decrease in adhesiveness.[69]

TSPP is able to form a crosslink with casein at pH 6, which can restrict the melt of processed cheese. In contrast, TSC would not crosslink with casein and an increase in pH augmented casein dispersion. The processed cheeses made using citrates and phosphate ES will exhibited an increase in degree of flow when the pH is raised from 5.3 to 5.6, possibly due to greater calcium binding by the ES, increased charge repulsion and greater casein dispersion.[46]

TSC also can contribute to greater meltability and sliceability of process cheeses compared to other ES; the process cheese also has a firm and gel-like structure.[35] TSC in combination with DSP at the rate of 3.0% level can produce significantly softer cheeses and more meltable than those prepared with condensed phosphate Joha salts. TSC can also produce the softest fat-free cheese spreads, which do have a meltability as compared to full-fat processed cheese spread made using DSP.[61]

3.7 PROCESSED MOZZARELLA CHEESE

Conventional mozzarella cheese involves some loss of milk solids while plasticizing of cheese curd required for stretching. To avoid such milk solids losses, processed mozzarella cheese has been prepared by adding 1.5% of ES (TSC + DSP; 2:1 w/w) to mozzarella cheese curd (ready for plasticizing) and directly processing it at 70 °C/15 min in a mechanical cooker, followed by molding and cooling. Such processed cheese had improved flavor, texture, and melting characteristics over cheese prepared by conventional method (i.e., plasticizing curd in hot molding water).[54] Processed cheese has been prepared by substituting young Cheddar (3 months) with mozzarella cheese up to 35.0% in the cheese blend, utilizing TSC at the rate of 2.0%, with attendant cost saving.[38]

3.8 REDUCED SODIUM PROCESSED CHEESE

The average sodium content has been reported to be 402, 335, and 382 mg per 28 g each of processed cheeses, cheese food, and cheese spread, respectively. The proposed sodium labeling for foods combined with a recommended dietary goal of substantially reducing sodium intake in several countries has focused attention on technological means to reduce sodium in processed foods. Use of tripotassium citrate, dipotassium phosphate, and tetrapotassium pyrophosphate has been reported to be useful in developing blends of ES for producing reduced-sodium processed cheese.[35]

ES such as potassium citrate (KC), potassium acetate (KA), and mixtures of KC + KA at ratios (i.e., 1:1, 2:1, and 1:2), at the rate of 3.0% by weight, were used in order to reduce the sodium content of cheese; control cheese was made using Kasomel 2394ES, that is, sodium citrate. Addition of potassium salts had little impact on chemical composition or sensory properties of the cheese spreads, but improved the Na/K ratio from 13:4 to 0:64 and the Ca/P ratio from 1:2 to 3:1. Satisfactory flavor and overall preference were associated with cheese spreads containing KC + KA at ratios of 1:1 and 1:2; such cheeses exhibited better emulsification properties than other samples.[1] The ES blend containing KC or potassium phosphate with usual sodium polyphosphate is necessary to be incorporated at just 0.89% level in preparation of satisfactory reduced-sodium block processed cheese.[36]

3.9 EMULSIFYING SALTS FOR CHEESE ANALOGS

The hot solution of calcium chelating salts are used to facilitate hydration of the protein, which subsequently acts as an emulsifier for the oil phase dispersed in the protein/aqueous phase of the cheese analog. The rheological properties of the rennet casein during hydration are highly dependent on the concentration of chelating salt in the hydrating solution.[27] In the manufacture of rennet casein based cheese analogs, calcium-chelating salts are used to disrupt the calcium mediated protein–protein cross-bridges and thus allow the protein to hydrate. Such improved hydration enhances the emulsifying ability of rennet casein in cheese analogs.[49] In the formulation of cheese analog, the type of ES used is dictated by the protein source used (i.e., rennet casein/acid casein/caseinates); the level of incorporation of ES decides the functionality and sensory properties of resultant cheese analogs.[44,51] The different stages of matrix development during manufacture of imitation cheese using ES blend (i.e., TSC + DSP) has been studied.[25,52]

In fresh cheese products based on acid casein, the ES are used to regulate pH, stabilize protein hydration and improve flavor, and keeping quality, whereas in most processed cheese products based on rennet casein (i.e., higher calcium content) their main functions are to regulate Ca^{2+}-Na^{+} exchange and prevent crystal formation.[64]

Sodium aluminum phosphate is commonly used in rennet casein/Na-caseinate based mozzarella type imitation process cheese since it provides desirable functional properties for pizza applications.[13,70] Trisodium and disodium phosphate were found to exert most pronounced melting effects, while Kasal (sodium aluminum phosphate) and Kasomet (blend of sodium metaphosphate and TSP) did not produce the desired melting effect in cheese product made by direct acidification.[41] Use of combined TSC + TSP (95:5) as ES at 3.0% yielded mozzarella cheese analog (MCA) with excellent shred, reduced fat leakage and better stretch as compared to the one made using TSC alone; meltability was impaired to some extent.[58]

Rennet casein powder, butter and ES were used to prepare spreadable processed cheese spread. Use of Joha C, Joha HBS, Joha C special, Joha S 80, Joha S 221, Joha S197, Joha RK2, and Joha PZ in cheese making can lead to variable pH, free oil, firmness, resistance to cutting, and meltability of the final products. Joha C salt gave cheese analog of acceptable rheological properties.[26]

A progressive increase in hardness and decrease in flowability of imitation cheese was observed when reduced quantity (up to 40.0%) of ES (TSC + DSP, 2:1) was employed as compared to normal usage level (i.e., 1.4% ES). Such reduction in the quantity of ES led to reduction in the mean fat globule diameter, decreased casein hydration and increased mixing times required to adequately emulsify the fat.[25] Addition of native starches to rennet–casein-based imitation cheese can alter the meltability and textural properties of the cheese. Hardness of imitation cheese can increase when wheat, potato, and maize starches are added, but it can be reduced when waxy maize and rice starches are added.

3.10 BACTERIOSTATIC ACTION OF EMULSIFYING SALT

Orthophosphate and polyphosphate ES may inhibit growth and toxin production by *Clostridium botulinum* (*Cl. botulinum*) by sequestering Fe, Mg, or Ca ions. Polyphosphates may also interact physically with bacterial cells by forming channels, increasing their permeability to inhibitory compounds and

promote leakage, and cell lysis. Growth and gas formation by *Clostridium tyrobutyricum* (*Cl. tyrobutyricum*) were prevented by use of 0.5–1.0% polyphosphate to process cheese. Several studies reported that sodium citrate had less inhibitory effect on *Cl. botulinum* growth compared to phosphate based ES.[40,62] Use of 0.5% long chain polyphosphate formulation (JOHA HBS-1, HBS-9) maybe sufficient to control *Cl. tyrobutyricum* growth under normal conditions, where initial spore counts are low and storage temperature is 20°C.[45]

The growth and survival rate of *Clostridia* were significantly reduced in cheese spread in presence of long chain polyphosphate mixture as compared to monophosphates. Use of long chain polyphosphate was suitable for the extension of shelf life of processed cheese spreads, when used at levels of ≥ 1.0%, even preventing blowing of cheese by *Clostridia* in accelerated tests.[65]

3.11 DEFECTS RELATED TO EMULSIFYING SALTS

If the ES are not utilized judiciously in cheese formulation, it may lead to some defects.

3.11.1 FISH-EYE DEFECT

"Fish-eyes" (hard, glassy lumps of protein) may be formed in processed cheese due to poor hydration of the protein.[6]

3.11.2 BITTERNESS IN CHEESE

An overdose of specific emulsifying agents (i.e., high in phosphorus content) can lead to bitterness in processed cheese slices. Such bitter slices showed very weak αs1- and β-caseins region, had only γ-casein and low-molecular weight peptides, and showed high concentrations of hydrophilic and hydrophobic peptides.[47]

3.11.3 CRYSTALS

Crystals of ES (i.e., calcium phosphate/diphosphate, calcium lactate/citrate) maybe observed in processed cheese as a result of: (a) an excess of ES, (b)

an unsuitable mixture of ES, or (c) insufficient dissolution of the latter in the mixture during processing.[11]

3.11.4 TEXTURAL DEFECTS

The texture may be rough or grainy, thin, pudding-like and the remedies suggested are to increase the level of ES, usage of Joha S9 or S10, and use ES with little ability to cream, respectively. In case of block cheese, defects like too firm or to loose are evident after packaging and to avoid such defects usage of ES like Joha C + T, C Special, S7, PZ salts are suggested. Spreadable processed cheese becomes gummy and sticky due to usage of ES, which are not suitable for creaming hence usage of ES like Joha S9 or Joha S10 is advocated.

3.11.5 OILY CHEESE

The defect may be attributed to usage of either too high or low amount of ES.

3.12 CONCLUSIONS

ES play a major role in determining the physical and chemical and to some extent controls the microbiological properties of processed cheese/cheese spread and even cheese analogs. The commercial blends of ES available in the market try to impose their monopoly in such specialty ingredient and are being sold at exorbitant price. Once the researchers come out with emulsifying combinations that "clicks" in the cheese system, it can be a "boon" to the cheese processors. Time has come to develop the possibilities of devising relatively cheaper and superior ES combinations specific to processed cheese or cheese analogs. Health conscious consumers are on lookout for "reduced sodium cheese products." Judicious use of proper type and concentration of ES is the key to success in cheese systems. Further research in this area seems to offer exciting prospects for development of different types of processed cheese/cheese spread and cheese analog specialties to tickle the palate of the varied consumers.

KEYWORDS

- **bacteriostatic**
- **casein**
- **cheese analog**
- **defects in cheese**
- **emulsifying salts**
- **functional properties**
- **Joha salts**
- **meltability**
- **oiling off**
- **processed cheese**
- **textural properties**

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PART II
Process Engineering—Dairy Products

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CHAPTER 4

DAIRY ENGINEERING: MILK PROCESSING AND MILK PRODUCTS

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CONTENTS

ABSTRACT

Milk is a perishable commodity and enriched in nutrients and requires immediate processing to extend the shelf life. Dairy products available in the market are fermented, heat desiccated, coagulated, clarified butter fat products, dried and frozen products. Processing can be carried out using thermal (pasteurization and sterilization) and non-thermal techniques (high pressure processing and pulse electric field). Different equipments involved during processing are chillers, centrifugal separator, homogenizer, microfluidizer, pasteurizer (batch and continuous), high hydrostatic processing machine and pulse electric field setup. Thermal mode of processing is well established and widely used but, nowadays non thermal processing is also getting recognition as consumer prefer minimally processes food with maximum nutrient benefits.

4.1 INTRODUCTION

Milk is an ideal food enriched with nutrients and is recommended by nutritionist for development of healthy body and consumed by all section of folks. Processing of milk is very important as it is perishable in nature. Both the reasons, that is, perishability and nutrient enriched (carbohydrates, proteins, fat, and vital minerals) makes milk vulnerable for spoilage due to proliferation of microorganism. To extend the shelf life of milk both thermal (pasteurization, sterilization, and ultra-high temperature) and non-thermal techniques (high pressure processing, pulse electric field and ultrasonic) are utilized. To process milk into various products, different types of equipment are used for example for converting liquid milk into milk powder different types of dryer are deployed. Packaging of milk contributes in preventing post process contamination and requires machinery like form-fill–seal machine for pouch milk or aseptic packaging for extended shelf life milk. Approximate composition of milk products is presented in Table 4.1. The worldwide total production, imports and exports of milk and milk products are mentioned in Table 4.2.

TABLE 4.1 Approximate Composition (%) of Various Milk Products.[6]

Product	Moisture (%)	Protein (%)	Fat (%)	Sugar (%)	Ash (%)
Acid whey	93.9	0.6	0.2	4.2	–
Anhydrous butter oil	0.2	0.3	99.5	0	0
Blue cheese	42.4	21.4	28.7	2.3	5.1
Butter	15.9	0.85	81.1	0.06	2.1
Camembert cheese	51.8	19.8	24.3	0.5	3.7
Casein powder	7	88.5	0.2	0	3.8
Cheddar cheese	36.7	24.9	33.1	1.3	3.9
Cottage cheese, creamed	79	12.5	4.5	2.7	1.4
Emmental cheese	36.0	28.9	30.0	–	–
Evaporated whole milk	74	6.8	7.6	10	1.5
Ice cream	60.8	3.6	10.8	23.8	1
Light whipping cream	63.5	2.2	30.9	3.0	0.5
Mozzarella cheese	54.1	19.4	31.2	2.2	2.6
Parmesan cheese	29.2	35.7	24.8	3.2	6.0
Processed cheese	39.2	22.1	31.2	1.6	5.8
Quarg	72	18	8	3	–
Skim milk powder	3.2	36.2	0.8	52	7.9
Sweetened condensed milk	27.1	7.9	8.7	54.4	1.8
Whey powder	3.2	12.9	1.1	74.5	8.3
Whole milk powder	2.5	26.3	26.7	38.4	6.1

4.2 PROCESSING OF MILK

Dairy industries use different methods for procurement of milk such as: by co-operative organizations, contractors, and individual producers/farmers. A detailed flowchart for processing of market milk is represented in Figure 4.1. The process of receiving milk at dairy is divided into several processing steps including unloading, grading, and sampling. Previously milk was collected and received in cans which were unloaded manually but nowadays most of the dairy industries receive the milk through tankers which are unloaded at the receiving bay using hoses and the milk is further pumped and stored into raw milk silos. After unloading of milk it is subjected for grading, and price fixing which can be done only on basis of fat present or on fat and SNF

TABLE 4.2 Production, Exports and Imports (Thousand Tons, Milk Equivalent).[5]

Country	Production			Imports			Exports		
	2010–2012 Average	2013 Estimate	2014 Forecast	2010–2012 Average	2013 Estimate	2014 Forecast	2010–2012 Average	2013 Estimate	2014 Forecast
China	41,879	44,919	45,252	8157	12,338	15,295	236	203	201
Developed countries	371,386	377,203	385,905	11,699	12,687	12,354	49,818	55,285	58,521
Developing countries	372,149	396,188	406,140	47,856	52,528	56,000	12,073	13,467	13,417
European Union	154,394	156,917	160,800	1383	1379	1467	15,533	15,808	16,677
India	127,382	138,093	144,860	334	60	75	296	1189	919
USA	89,118	91,210	93,939	1375	1430	1440	8358	10,412	11,013
World	743,535	773,391	792,045	59,555	65,215	68,354	61,891	68,752	71,939

content. Platform test are used to grade the milk by evaluating milks smell, appearance, sediment, acidity, and lactometer reading.

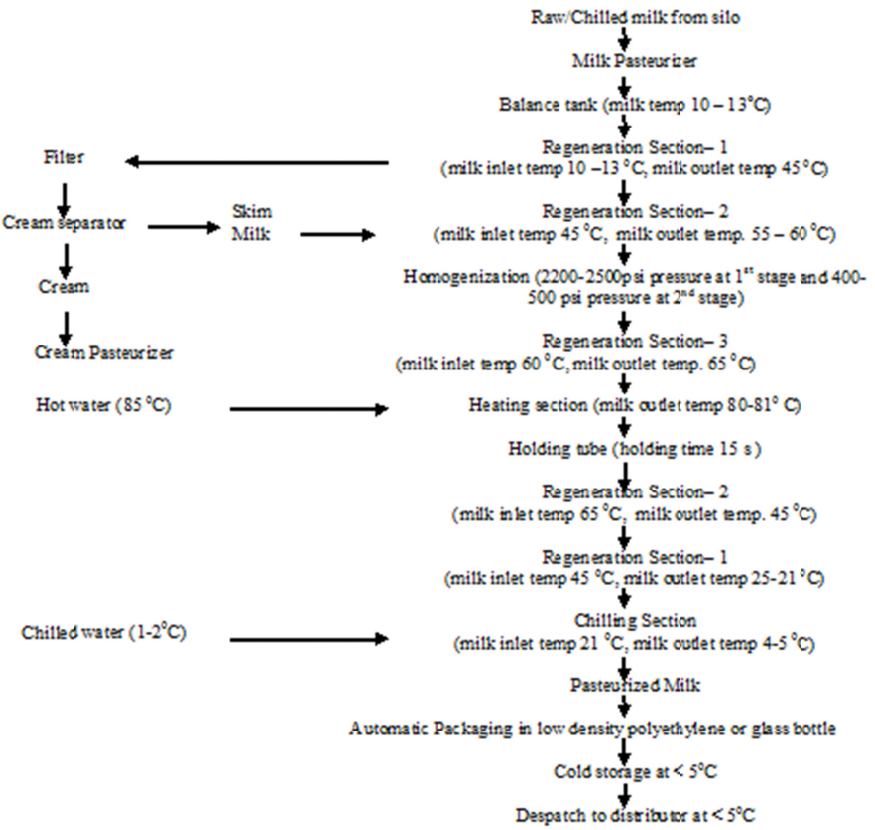

FIGURE 4.1 Processing of market milk.

4.2.1 EQUIPMENTS AT COLLECTION AND STORAGE CENTERS

Earlier practice was to collect the milk from nearby farms/small dairies were controlling the sector so there was no requirement for installation of chilling facility and other storage equipments. However, with set up of large dairy processing units, co-operative societies and increase in distance between collection and processing center hence chilling centers were started by the dairies at geographically strategic locations and milk shed areas. Milk can be collected directly from producers and is stored in chillers below 5 °C. Cooling can be achieved by immersion, in-can cooling, brine cooling, bulk

milk cooler, rotor freezer, plate chillers, and tubular coolers. Cooling of milk prevents proliferation of microorganisms and hence prevents further spoilage. Until milk is further utilized for processing, it is stored in insulated tanks/silos after assessing the quality at less than 4 °C. Two types of silos are generally used in the dairy industry, one are those which are smaller in capacity (5000–60,000 L) and can be located indoors and the second type are larger (100,000–150,000 L), which are placed outdoors. Silos are made up of two layers of stainless steel and between these layers insulation material is sandwiched.

Longer storage periods of milk in silos at less than 4 °C causes separation of cream, hence the modern silos used in dairy industry are fitted with agitators which prevent the gravity separation of cream (Fig. 4.2). Agitation process must be smooth, as too violent can cause disintegration of fat globules rendering them susceptible for enzymatic activity of lipase which causes off-flavor. To assist the smooth functioning of the processing section and maintain the quality and quantity of the milk, silos are fitted with indicators for temperature, high and low level of milk and sampling cocks to withdrawing the sample.

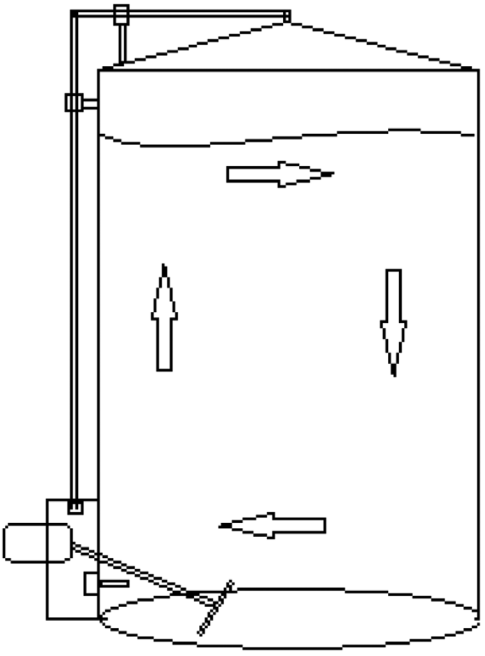

FIGURE 4.2 Silo tank with agitator.

4.2.2 CLARIFICATION, SEPARATION, AND STANDARDIZATION

Clarification is a process in which milk is subjected to centrifugal separation in which the visible foreign matter can be separated. In a centrifugal clarifier the milk is passed through the channels toward the axis of rotation, and the clean milk leaves through the outlet situated at the top and the foreign material and dirt are separated out through the periphery of the clarifier bowl.

The first unit operation in the processing of market milk is cream separation. Cream is present in the form of solid fat in raw milk and diameter of fat globules can range from ~2–20 μm. For centrifugal separation of cream from milk various separators are available which include warm milk separator, cold milk separator, power driven separator, hand driven separator, semi-enclosed separator, airtight separator, and disc bowl separator. Generally disc bowl separator is used for separation of cream in most of the dairies. Disc bowl separator uses centrifugal force to separate cream and the appearance and construction, is quite similar to clarifier but with certain differences which include: (a) separator do have two outlet (one for low density cream and another for skim milk (Fig. 4.3) while clarifier have only one outlet, (b) diameter of disc clarifier bowl is smaller than separator, and (c) clarifier has open holes at the outer edge of the disc but separator has open holes at the periphery.

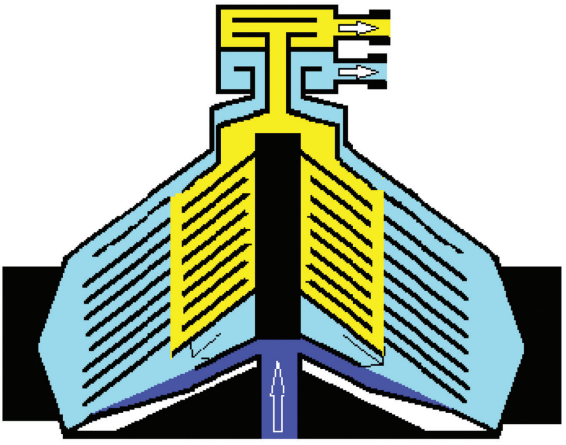

FIGURE 4.3 Centrifugal separator bowl.

Separation of cream in cream separator is based on Stokes' law:

$$V = \frac{r^2 \left(D_s - D_f\right) N^2 .R.K}{n} \tag{4.1}$$

where, V = velocity of movement of a single fat globule, r = radius of the fat globule, D_s = density of skim milk, D_f = density of fat globule, N = speed of the bowl (RPM), R = distance of fat globule from the axis of the rotation, K= constant, and n = viscosity of skim milk.

Speed of the separation of cream can be increased by increasing the diameter of fat globule, greater difference in the diameter between skim milk and fat, greater speed or size of bowl or lower viscosity of skim milk. The skimming efficiency of the cream is the percentage of total fat in the cream recovered from the milk.

The next processing step in the market milk is standardization which is adjustment of the fat/SNF content of milk/milk product by incorporation of cream/skim milk. Generally, standardization of raw milk is carried out before it is processed further, and also depends upon regulatory policies of different countries as the step is not permitted in many countries. The relative quantity of the materials involved in the standardization may be calculated with the help of Pearson's square method in which the desired fat content is placed at the center of the square. In the initial step subtract the supplement prerequisite from the supplement fixation (on left of square) in the feed across the diagonal (upper left – center = base right; base left – center = upper right). Practice this for both sustains. Make any negative numbers on the right half of the square positive. The answers on the right half of the square are the parts of milk/cream/skim milk to incorporate in the proportion. After subtracting across the diagonal, sum the parts of the two feeds to get the total. Then, divide each part by the sum of the parts to calculate the percent of each feed in the proportion.

Pearson's square method is used by many dairies but due to advancement in technology it is not much practiced. Depending upon level of automation and mechanization in dairy industry, standardization of milk is either done direct in-line or batch standardization is conducted. Nowadays dairy industry discourages manual/batch standardization due to hygienic problems and increased volumes make manual/batch standardization a cumbersome process, so direct in-line standardization are deployed. Direct in-line standardization is used for standardization of fat as well as protein. During fat standardization in direct in-line, standardization method for the cream separator is also in-line, which helps to standardize the milk without any delay (Fig. 4.4).

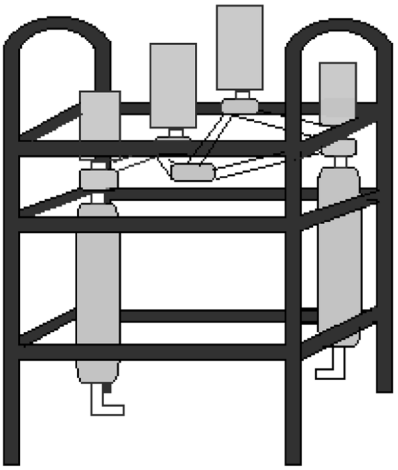

FIGURE 4.4 Direct in-line standardization system.

Direct in-line standardization system consists of control valves, flow and density meters, and a computerized control loop, which is used to adjust the fat content of milk to predetermine values. The fat content of milk is inversely proportional to the flow rate and flow meters are installed in the unit to determine the flow rate. For standardization of protein, a protein sensor is attached with this unit, which determines the actual protein content in the standardized milk. By joining this unit with a source of protein, it is conceivable to standardize both the fat and the protein content at the sometime.

4.2.3 HOMOGENIZATION

Homogenization of milk is carried out for breaking down the fat into smaller particles by pumping milk at high pressure through a small orifice, called valve. Homogenization increases the surface area of fat globules significantly as a result of breakup of larger globules.[1, 3, 7] In homogenizer the milk is passed through orifice of 0.1 nm to break the fat globules (<2 microns). Break down of fat globules is achieved by combination of turbulence and cavitation caused during passage of milk through value at a high pressure which does not allow visible cream separation during its storage. Homogenization also enhances the mouth feel, digestibility, and color of the milk. Normally, homogenizers used at industrial scale are either single-stage

(2500 psi) or two-stage (1st stage at 2000–2500 psi and 2nd stage at 500 psi). Generally milk homogenized with single-stage homogenizer is more viscous, whereas two-stage homogenizer is applied when high homogenization efficiency is desired. Advantages and disadvantages of homogenization of milk are described in Table 4.3. For boosting the pressure of milk, a piston pump is attached which forces the milk smoothly and uniformly distributes the milk into the seat gap. The homogenization pressure is adjusted by controlling the gap between the forcer and seat with the help of a hydraulic actuator (Fig. 4.5).

TABLE 4.3 Advantages and Disadvantages of Homogenization of Milk.

Advantages	Disadvantages
• Increases smoothness/creaminess of the milk. • Non-formation of skim or cream layer during storage for a long time. • It increases the surface area of fat globules and in return helps easy digestibility. • Provides brighter appearance than un-homogenized milk. • It produces soft curd, hence it may be recommended for infant feeding. • Slighter increase in viscosity of milk.	• Cost of production also increases marginally. • Fat cannot be separated from homogenized milk. • Due to breakdown of fat globules more surface area is available for microbial contamination. • More susceptible to produce sun induced flavor due to the conversion of methionine to methional. • It may induce bad flavor by increasing the chance of lipolysis due to lipase enzyme (Lipoprotein lipase is a heat labile enzyme and its effect may be reduce by pasteurization before or instantly after homogenization).

The efficiency of the homogenization may be a helpful indicator for monitoring the adequacy of homogenizer. Homogenization efficiency of homogenizer can be determined by leaving 100 mL of milk in a measuring cylinder undisturbed for 24 h in an icebox and determining the difference in fat content in the upper layer and the remaining milk by Gerber method. According to Wilbey,[8] homogenization efficiency is expressed as a homogenization index:

$$\text{Homogenization Index} = \frac{\text{Difference in fat contents}}{\text{Fat value in the upper layer}} \times 100 \qquad (4.2)$$

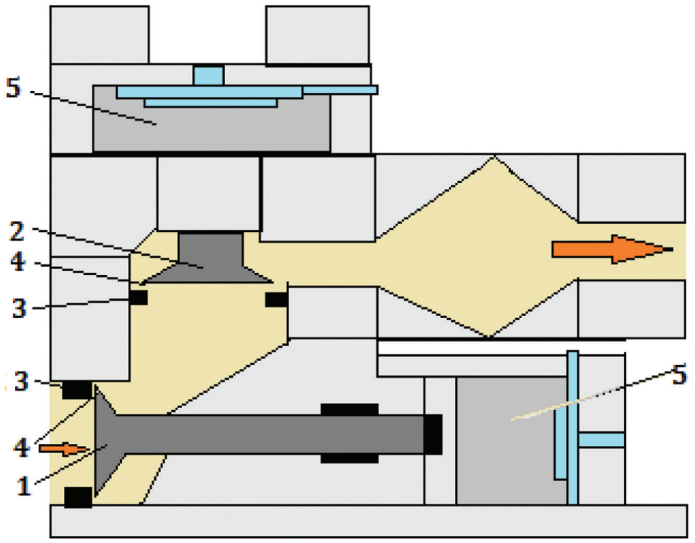

FIGURE 4.5 Two stage homogenizer: where, 1 = First stage forcer, 2 = Second stage forcer, 3 = Seat, 4 = Gap, and 5 = Hydraulic actuator.

There are several applications of homogenization in different industries such as: dairy/food industry, pharmaceutical industry, biotechnology, and chemical industry. For achieving homogenization, other devices have become very popular like mixers, colloid mills, microfluidizers, ultrasonic homogenization, and membrane emulsification. In mixing devices, rapid mixing and blending equipments are the most regularly utilized for emulsifying fats or oils into a fluid stage in the dairy industries. A stirrer with different designs is attached with mixing device and due to the rapid rotation it creates inclinations in milk which bring about the disturbance of the oil–water interface, bringing about the emulsification. In colloid mills, a rotator disc and a stationary disc are attached and the milk passes through a narrow gap between these two rotators and produces a shear stress, due to this shear stress larger milk fat globules are disrupted they are broken down into smaller ones.

Microfluidization is a novel technique that breakdown the fat globules at very high pressure. Disintegration in a microfluidizer of fat globules is carried out by combination of high velocity, turbulence, shear force, and collision between milk molecules in the interaction chamber. It helps to emulsify and uniformly distribute the fat globules in the milk within a span of 5 s. Due to high shear and impact forces not only fat molecules are broken down but

protein molecules disrupts and both of them produce a complex structure which helps in the formation of firm texture in products like yoghurt[2] and other dairy products like cheese.

Ultra sound of frequency (<100 kHz) has also been used in some dairy industries for homogenization of milk. In ultrasonic homogenization, extraordinary shear and pressure gradients are caused by high intensity ultrasonic waves which disrupts the larger fat globules into smaller ones. Generally for dairy industry ultrasonic homogenization is conducted within a frequency of 20–50 kHz.[6] Ultrasonic homogenization has the capacity to produce nano-emulsions with almost the same emulsion size as the micro-fluidizer, however sonication delivered emulsions with more wider and bimodal size distribution.

4.2.4 PASTEURIZATION

It was demonstrated by Louis Pasteur in 1860 that heat treatment at 50–60 °C can kill most of the spoilage causing microorganism. Milk pasteurization refers to treating each particle of milk with a suitable time–temperature combination, which is effective to destroy bacteria (*Coxiella burnetii, Mycobacterium tuberculosis, and Listeria monocytogenes* as indicator) with least effect on color, flavor, and nutritional component of milk. The aim of pasteurization is to increase the shelf life by killing the non-spore forming pathogenic bacteria and maintain the keeping quality by reducing the enzymatic action especially of lipase. In pasteurization, destruction of micro-organism follows the first-order kinetic reaction: When a logarithmic plot is plotted between time and surviving of micro-organism population, a linear graph (straight line) is obtained. The decimal reduction time (D) is the time in which 90% reduction of bacteria occurs in one log cycle and the value is the independent of the size of the microbial population but it depends on the temperature. Temperature dependence of D value is calculated with the help of z value (in °C or °F). The Z value is defined as the degree of temperature causing a decimal change of D value and alkaline phosphatase test is usually used as an indicator to check the pasteurization efficiency. Milk is pasteurized in dairy either by a batch or continuous process (Fig. 4.6). Generally, continuous types of pasteurization system are used in dairies.

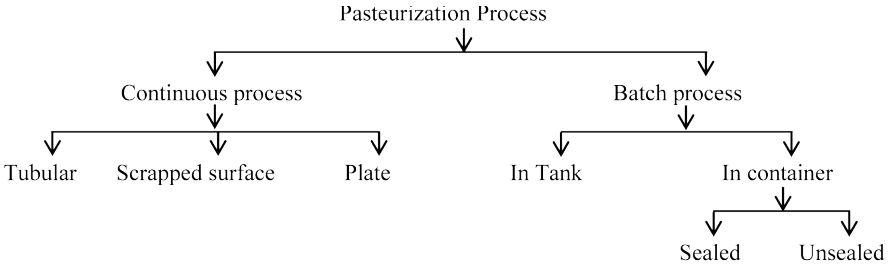

FIGURE 4.6 Types of pasteurization process.

4.2.4.1 BATCH TYPE PASTEURIZATION

Batch type pasteurization is a simplest method in which milk (~1000 L) is heated and possibly cooled within a jacketed vessel or attached with heating coil. Batch type pasteurizers are usually operated at *low temperature for longer time* (LTLT, 63 °C/30 min). Two types of batch-pasteurizer are usually used at low scale: (a) in-tank pasteurization and (b) in-container pasteurization, which is further available for unsealed and sealed containers. Some of the advantage and disadvantages associated with batch type pasteurization are described in Table 4.4.

Batch type pasteurizer is normally made up of a jacketed vessel fitted with an agitator and a temperature sensor and a source of heat. Control panel maintains the time and temperature of milk and ice-cream mix for 30 min at 63 and 66 °C, respectively for pasteurization.[9]

TABLE 4.4 Advantages and Disadvantages of Batch Pasteurization.

Advantages	Disadvantages
• Simpler method for pasteurization of milk.	• Heat transfer rates are low.
• Lower capital cost.	• Thermal efficiency is generally low.
• Useful for small-scale dairies and operations.	• Slow heating and cooling rates for a long time may increase the high level of chemical changes.
	• Product specific.

4.2.4.2 CONTINUOUS TYPE PASTEURIZATION

Continuous type pasteurization is operated at *high temperature for short time* (HTST, 72 °C/15 s) and immediately cooled below 5 °C. In this type of pasteurization, heat exchanger is used to maintain the temperature and continuous flow of milk. Basically three types of heat exchangers are use in the dairy industries: plate heat exchanger, tubular heat exchanger, and scraped-surface heat exchanger.

In plate heat exchanger, two plates are separated by a thin elastomeric seal through which milk flows and its surface to volume ratio is 500:1. In plate heat exchanger, rippled plates are used due to this flow is separated and turbulent flow can be kept up at moderately low speeds over a large surface area (Fig. 4.7). Advantages and disadvantages of implementing plate heat exchanger for pasteurization of milk are described in Table 4.5.

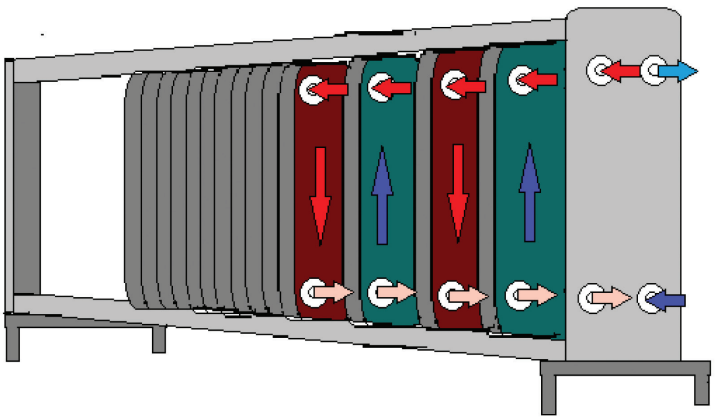

FIGURE 4.7 Plate heat exchanger process.

Tubular heat exchangers are usually deployed for processing highly viscous liquid food products, whereas plate heat exchanger is used for low viscous fluid products (Fig. 4.8). Path width of tubular heat exchanger is greater (~5–10 mm) with respect to that of plate heat exchanger (1.5–5 mm). Hence tubular heat exchanger may become an alternative to plates heat exchanger, where fouling is a major problem.

Scraped-surface heat exchangers are used for heating and cooling of highly viscous, sticky, and lumpy food products (Fig. 4.9). Two or more scraped-surface heat exchanger may be connected in series or parallel to increase the capacity of the plant and obtain a high heat transfer surface.

TABLE 4.5 Advantages and Disadvantages of Plate Heat Exchanger of Milk.

Advantages	Disadvantages
• Occupies less floor space.	• Leakage chances are more if not properly maintained and serviced.
• Overall heat transfer coefficient is high in comparison with batch process.	• Running cost is slightly higher as compared to batch pasteurizer.
• PHE is made up of S.S. pressed plates, which helps in resistance to corrosion.	• Particulate food products can cause blockage of the PHE as the spacing between two plates is as low as 3 mm.
• Ease in cleaning and adaptability for cleaning-in-place systems.	• Not useful for high viscous fluid.
• PHE can operate even at small temperature differences.	

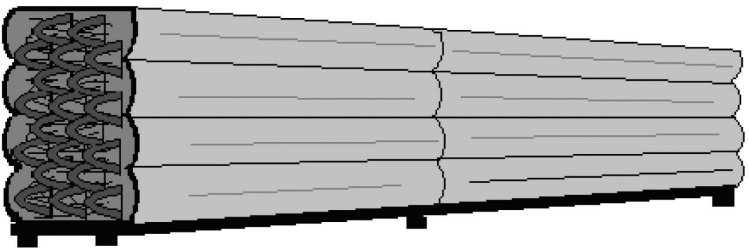

FIGURE 4.8 Tubular heat exchanger.

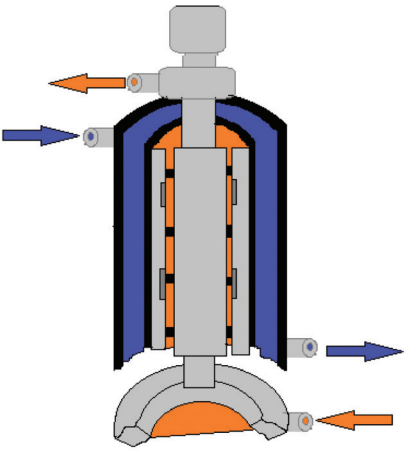

FIGURE 4.9 Scraped surface heat exchanger process: Orange color shows flow of product and blue color shows the flow of heating or cooling medium.

4.2.5 ULTRA-HIGH TEMPERATURE (UHT) TREATMENT

Heating of milk at temperature higher than 135 °C for a very short time (few seconds) is referred as *Ultra-High Temperature* (UHT) Treatment. UHT treated milk products are immediately cooled followed by aseptically packaging in sterile containers to achieve commercial sterile milk products. During long storage (6 months to 1 year) at room temperature, there are chances that fat may be separated, hence in UHT processing; homogenization of milk is performed and operated under aseptic conditions. Some of the advantages and disadvantages associated with UHT treatment of milk are depicted in Table 4.6.

TABLE 4.6 Advantages and disadvantages of UHT processed Milk.

Advantages	Disadvantages
• Availability of milk throughout the year due to its longer shelf life. • Refrigeration facility is not required for storage of UHT processed milk products. • Lower transportation cost. • Less chances of spoilage. • Lower work force is required. • Convenience for consumer.	• Packaging and processing cost increases the total cost of milk. • After processing, milk products often have a strong cooked flavor, which reduces the consumer acceptance.

Direct heating and indirect heating are two major heating mediums used in UHT processing of milk. **In direct heating method**, milk comes into direct contact with the heating medium that is steam. While in **indirect heating method**, milk and steam are separated out with the help of stainless steel plate/pipe. In direct heating method, heating and cooling cycle occurs at a very rapid rate as compared to indirect treatment and the reason is attributed to the transfer of latent heat of condensation and evaporation, respectively, between steam and milk. Plate heat exchanger is often used in the direct UHT processing of milk. When direct heating method is used, sterilization temperatures are 3–4 °C more than indirect heating so that equal sterilization effect can be achieved as greater heat input is required during the heat-up phase. Compared to direct heating systems, indirect heating systems have low heat transfer rate and higher rate of deposition over heating surface, which demands frequent cleaning of the sterilizer. Indirect systems require less control, less processing and operational cost (less pumps and accessories, regeneration of energy) than direct systems for sterilizing milk.

4.2.6 STERILIZATION

In sterilization process, milk is heated to above 100 °C or with a time–temperature combination (121 °C/15 min) at 15 psi pressure. The sterilization process may be a batch (autoclave) or continuous process (vertical hydrostatic towers or horizontal sterilizer) and can be carried out either in cans or glass bottles, which are prefilled and hermetically sealed. Sterilized milk is heated to above 100 °C for a sufficient length of time to satisfy the turbidity test, which identifies the degree of denaturation of whey protein especially for β-lactoglobulin, α-lactalbumin, and blood serum albumin. Turbidity test is two-step process: **In the first step**, precipitation of casein and denatured whey protein is caused by the addition of ammonium sulfate. **In the second step,** the filtrate is boiled for 5 min. Residual material should be absent in sterilized milk, which indicates the adequacy of sterilization process.

4.2.7 NOVEL NON-THERMAL MILK PROCESSING TECHNIQUES

Consumers prefer products that are minimally processed, having natural aroma, fresh taste, additives free, stable, convenient to use, and microbiologically safe. Although thermal processing (heating or cooling operation) maintains safety of food but it adversely affects the nutritional value. Then irradiation, pulse electric field, and HPP are alternatives for milk processing. Technologies such as ohmic heating, dielectric heating (which includes microwave heating and radio-frequency heating), and inductive heating are being developed. Novel technologies hold a common feature, that is heat is generated directly inside the food and which has direct implications in terms of both energetic and heating efficiency. Among these non-thermal techniques high-pressure processing of milk has been well studied although other techniques have attracted little commercial application but do have a considerable potent future.

4.2.8 HIGH HYDROSTATIC PRESSURE PROCESSING (HPP)

HPP is a novel non-thermal technique that utilizes pressure (100–900 MPa), which is instantaneously and uniformly transmitted throughout the product irrespective of its geometry. It relies on the Le-Chatelier's principle and isostatic transfer.[9] HPP of milk has attracted attention of the consumers as it has least effect on flavor, color and nutritional value, simultaneously very

effective to reduce microbial growth. The equipment comprises of pressure vessel known as heart of vessel, closures at top and bottom, pressure intensifier, system for controlling process and instruments to load and upload the products. The pressure vessel can be vertical or horizontal in nature. HPP can be a batch process, semi-continuous or continuous. In batch system, product is packaged before pressurization, and compression and decompression cycles are operated in same vessel. The material used for packaging of food products must bear 15–20% compression. In semi-continuous or continuous process, flow is continuous and compression is in one vessel and decompression takes in another vessel. Pump is required to introduce the pressure transmitting liquid in the vessel and then pressure is applied till desired level of pressure is reached. Liquid usually used in the pressure vessel is generally distilled water but on many occasions silicon oil, propylene–glycol mixture, and castor oil are also used in HPP. This non-thermal technique offers the dairy sector to produce microbiologically safe and minimally processed products and also to develop novel dairy products.

4.2.9 PULSE ELECTRIC FIELD (PEF) PROCESSING

PEF is also a non-thermal technique that has potential to increase milks shelf life and had shown minimal alteration on the nutritional and other functional characteristics. It is also effective to inactivate microorganisms responsible for spoilage. PEF is based on the application of pulses of high voltage (typically 20–80 $kVcm^{-1}$) delivered to the product placed between a set of electrodes that confine the treatment gap of the PEF chamber.

4.3 TYPES OF MILK PRODUCTS

During glut season, milk can be processed into different products to make it available around the year and in the lean season. Pasteurized milk, sterilized milk, and homogenized milk are processed by using a set of equipments including pasteurizer, sterilizers, and homogenizers. Special milks (whole milk, standardized milk, toned, double toned milk, flavored milk, soft curd milk, and vitaminized/irradiated milk), fermented milk (natural buttermilk, cultured buttermilk, acidophilus milk, kumis, and kefir), soya milk, filled milk, reconstituted/rehydrated milk, recombined milk, and imitation milk are generally pasteurized/sterilized or UHT treated (Table 4.7). Milk products available in the market are: cream, butter, butter oil, cheese, ice cream,

condensed milk, powdered milk, and so forth. Manufacturing of these products varies all over the world as a result of traditional practices, variation in dietary habits, religious dissimilarities, and scale of atomization. In dairy market, liquid milk (54%) occupies largest segment followed by ghee, yoghurt, and whiteners 14, 8, and 4%, respectively.

TABLE 4.7 Classification of Milk and Milk Products.

Milk products	Classification of milk products	Example
Fermented products	Yoghurt	Stirred yoghurt; low fat yoghurt; sweetened or flavored; strained yoghurt; frozen yoghurt
	Fermented milk products/ Beverage	Kumiss; kefir; acidophilus milk; bulgarian buttermilk
Heat desiccated products	Concentrated milk	*Khoa* (*burfi*, *peda*, *gulab jamun*)
	Condensed milk	Sweetened condensed milk; sweetened condensed skim milk; unsweetened condensed milk; unsweetened condensed skim milk
	Clotted cream	
Coagulated products	Cheese	Pressed cheese (mimolette); semi-pressed cheese (cheddar); pressed and cooked cheese (gruyère); hard (cheddar); medium-hard (gouda); soft (port salut)
	Chenna	
Fat rich products	Butteroil	
	Butter	
	Ghee	
Frozen products	Ice-cream	Low-fat ice-cream, medium-fat ice-cream, soft serve ice-cream
Dried products	Whole milk powder	
	Skim milk powder	
	Butter milk powder	
	Whey powder	
	Butter powder	
	Ice-cream mix powder	
	Cheese powder	
	Infant milk powder	

KEYWORDS

- **aseptic packaging**
- **butter fat**
- **centrifugal separator**
- **chiller**
- **high hydrostatic**
- **processing**
- **homogenizer**
- **homogenization**
- **microfluidizer**
- **milk**
- **nutrient benefits**
- **pasteurizer**
- **perishable commodity**
- **plate heat exchanger**
- **pulse electric field**
- **scraped surface heat exchanger**
- **shelf life**
- **sterilization**
- **Stokes' law**
- **thermal mode of processing**
- **ultra-high temperature**

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CHAPTER 5

ASEPTIC FOOD PROCESSING AND PACKAGING

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ABSTRACT

The APP of milk and milk products broadens the use of packaging materials to achieve a shelf stable product at room temperature. The primary motivation for the development of the UHT processing and aseptic packaging technology was to achieve a higher-quality product with the same level of microbiological safety as attained in traditional canning processes, lower production costs, increased convenience, continuous production, and lower nutrient loss. However, heat preservation requirements for food safety can and will affect quality. The more severe a sterilization process, the greater will be the degradation of food quality, both in consumer appeal and in nutritional factors, hence the UHT processing and aseptic packaging can be implemented. Indirect heating systems along with paperboard carton forming and packaging machine is the widely used technique for milk and milk products.

5.1 INTRODUCTION

Aseptic processing and packaging (APP) is a very well established technology in food service applications worldwide as a safe and high-quality packaging option for foods. The demands of high quality shelf stable and safe food has created huge development of APP of foods.[35] Technological advances in designing high-speed processing and packaging equipments aid boosting the use of aseptic packaging technology nowadays. Challenges of aseptic processing include: post-process contamination, handling and storage of aseptic pack, customer acceptance and heat induced chemical/physical changes and extended storage up to 12 months depending upon the type of product packed. Packaging integrity and maintenance of sterility, package and equipment sterilization techniques are active areas of research and development. Despite the complex system of APP system, the high quality of the end product will make this technology more prevalent in the world market as consumers are becoming more conscious about the safety and quality of foods to lead a healthy life.

APP involves *Ultra-High-Temperature* (UHT) sterilization of a food product followed by holding it for a specified period of time, cooling and finally packaging in pre-sterilized containers under aseptic condition. UHT sterilization is defined as continuous flow sterilization processes, which employ heat treatments within the temperature range of 130–150 °C with holding time of 2–8 s. The upper end of the temperature range tends to be

used for low viscosity products such as milk, and the lower end for more viscous products.

A schematic representation of an APP system is shown in Figure 5.1. A typical APP system consists of product sterilizer, filler and ancillary piping and filler bowl, sterile gas lines and filters, sections where containers are formed/erected and lid materials are sterilized and aseptic zone where containers are filled and sealed. The term *"aseptic"* implies the absence or exclusion of any unwanted organisms from the product, package or other specific areas, while the term hermetic (strictly air tight*)* is used to indicate suitable mechanical properties to exclude the entrance of micro-organisms into a package and gas or water vapor into (or from) the package.

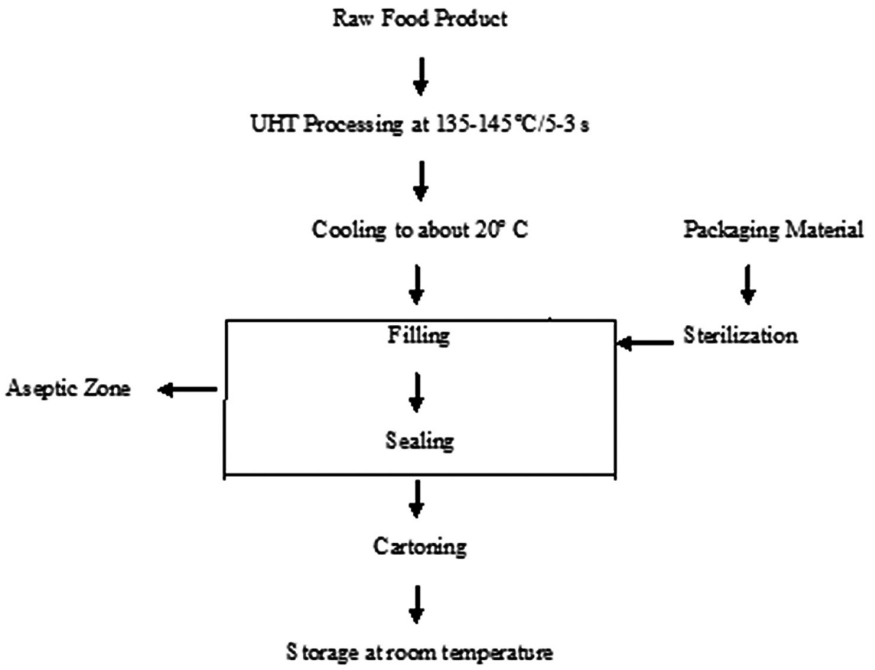

FIGURE 5.1 Schematic flow diagram of aseptic processing and packaging system.

Aseptic processing also preserves nutrient and sensory attributes of a food product besides commercial sterility. Commercial sterility is defined as a condition that renders a food free of microorganisms (including spores) capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution. APP offers advantages to the consumer as

well as to distribution channels (lower distribution and storage costs, more appropriate shelf life, relief of pressure on chilled cabinet, cost effective, and free from additives). The task of the aseptic packaging operation is to maintain the high microbiological quality of the product for the length of its intended shelf life and retain consumer acceptance with regard to flavor, texture, and nutritional value of the product during the promised shelf life. Types of dairy products which are processed by UHT treatment followed by aseptic filling include whole milk, flavored milk, cream, concentrated milk, recombined milk, ice-cream mixes, puddings, and whey based drinks.[8] Apart from these, fermented milk products, yoghurt, butter milk, desserts (custards and puddings), protein drinks, soy drinks, nutritional solutions, baby foods, fruit and vegetable juices, beverages such as tea and coffee, toppings and creams based on vegetable fat, soups, sauces, purees, dressings, and so forth are also processed using UHT treatment.[46]

Aseptic milk and milk-based products can be stored on the shelf for up to six months without refrigeration. The demands of high quality shelf stable, and safe food has created huge development of APP.[29,33,40,41] Aseptic packaging has had only limited success in food markets, due partly to technical difficulties in processing and filling particulate products, which have individual and varied handling requirements but is of major importance in the milk and milk products for extending the shelf life. Europe had the largest share in the UHT milk market in 2012, but the market growth in Asia Pacific is expected to make it the global leader by 2019. The key domestic companies in some of the prominent UHT milk markets are: Parmalat, Candia, and Dairy Partners Americas. On the other hand, the global players include Nestle, Lactalis, and Fonterra.

At present only 1–2% of total milk produced in India is UHT treated and packed aseptically and is currently growing at 28–30% per annum. The packed juices (6% of the total juice drinks market) and nectars market in India is around 33.90 million liters out of which 20.8 million liters is processed and packed aseptically. With growing population, changing food habits and increase in incomes, the demand of aseptic products are steadily increasing. UHT processed foods are widely used in many countries of the world. Two of the more recent applications of aseptic technology are bulk storage and transportation, and shelf stable products. The most common aseptic packaging is brick-shaped cartons. The main limitations of APP are: the initial capital cost, highly skilled operators, and complexity of the plant, which arises from the necessity to sterilize packaging materials, pipelines, storage and working tanks, filling area, surfaces in filling machine, and maintenance of sterile air.[19]

5.2 HISTORY OF ASEPTIC PROCESSING AND PACKAGING

The first aseptic packaging of food (specifically milk in metal cans) was carried out in Denmark by Nielsen before 1913. In 1933, American Can Company for filling liquid and semi-liquid foods developed a machine, which used saturated steam under pressure to sterilize the cans and ends known as heat–cool fill (HCF) system. The product to be filled in the sterile can using HCF system was heated (150 °C/15 s) followed by immediate cooling.[5] Real progress in the commercial development began with the invention of the Dole–Martin process in the late 1940s in California by the Dole Engineering Company.[28] Dole–Martin process consisted of product sterilization using a tubular heat exchanger, can sterilization using superheated steam at 210 °C, filling of product under aseptic conditions and usage of superheated steam to sterilize the environment in sealing section.[32] The Dole Company bought the first commercial aseptic filling plant in the market in 1950. The first milk with a long shelf life packaged in paperboard laminate was sold in Switzerland in October 1961.[42]

The Avocet system was used for UHT processing of cream and filling into sterile glass bottles in 1950s while in 1950s, Tetra Pak introduced tetrahedron package primarily for milk and liquid dairy products. The steam-into-milk UHT system was available commercially around 1950. The first commercial aseptic plant was built in 1951 in Washington, USA by Roy Graves and Jack Stambaugh and was built on one of the fundamental aseptic canning machines used by Dole process. This development was the starting point for expanding to different package types and sizes. The first commercial production of UHT milk was undertaken only in 1961 in Switzerland by Tetra Pak. In the early 1960s, the Tetra Pak became the first company in the world to market aseptic packaging technology and more than 80% of the aseptically packed products are from Tetra Pak systems.

In India, the first UHT sterilization plant for milk was commissioned at Surat in 1982 under Operation Flood Program.[13] The Combibloc aseptic system was developed in late 1970s, which used carton blanks instead of roll stocks and after this, aseptic filling in drums and bag-in-box fillers were established. The use of hydrogen peroxide for the sterilization of packaging surfaces was approved by the FDA in 1981.[7,15] The aseptic filling of non-carbonated beverages in cans was started in 2003 in France. UHT processes and aseptic filling of food products including milk and milk-based products is now being practiced throughout the world particularly in the countries where maintaining cold chain is expensive.

5.3 ASEPTIC PROCESSING KINETICS

The kinetics of thermal inactivation deals with the destruction of spoilage causing microorganisms and enzymes. The kinetics of thermal inactivation normally follows a first order chemical reaction which is given by the equation:

$$\frac{dN}{N} = -kN \tag{5.1}$$

Where, N is the number of spores surviving after time t (s) and k is the death rate constant (s^{-1}). Integration of Eq 5.1 from time 0 to time t yields:

$$\frac{N}{N_o} = \exp(-kt), \text{ or} \tag{5.2}$$

$$\log_{10}\left(\frac{N}{N_o}\right) = \frac{-t}{D} \tag{5.3}$$

where, N_0 is the number of spores at time $t = 0$, $D = 2.303/k$.

D is the decimal reduction time, which is the time required to reduce the number of surviving spores by 90 or 10% survival.[24] This can be determined from heat resistance experiments by plotting "log N" against "t" using the equation:

$$\log N = \log N_o - \frac{t}{D} \tag{5.4}$$

The D value determined at a reference temperature (T_{ref}) is denoted by D_{ref}. The value of decimal reduction time D has been found to vary with temperature according to the equation given below:[6]

$$\log_{10}\left(\frac{D}{D_{ref}}\right) = \frac{T_{ref} - T}{z} \tag{5.5}$$

where, D is the decimal reduction value at temperature T and z is the change in temperature required to change the decimal reduction value by 90%.

The ratio of D_{ref} to D is referred to as the lethal rate (L). F-value for a process is defined as the processing time at any temperature to achieve a

certain level of microbial kill. *F*-value can be computed in terms of lethal rate by the equation given below:[4]

$$F = \int_0^1 L dt = \int_0^1 10^{\frac{T_{ref} - T}{z}} = D_{ref} \log_{10} \frac{N_0}{N} \tag{5.6}$$

The *F*-value at a reference temperature of 121.1 ºC (250 ºF) and a *z* value of 10 ºC (18 ºF) is referred to as the F_0-value. The F_0-value required to achieve a 12-log cycle reduction of *Clostridium botulinum* in a low-acid food product is 3 min, which indicates that the process is equivalent to a heat treatment of 3 min at 121.1 ºC. Thus, many combinations of time and temperature can yield an F_0-value of 3 min. The ratio of F_0-value of the process to the F_0-value required for commercial sterility is known as lethality and it must be at least unity for commercial sterility in aseptic processing.

The destruction of nutrients and inactivation of enzymes follow similar kinetics to that of the destruction of microorganisms. The quality advantage that accrues from the use of UHT processes is compared by *z*-value of microbial destruction with the *z*-value for the loss of desirable quality factors in the food such as nutrients. Destruction of nutrients during thermal processing is quantified by cook value *C,* which is similar to the definition of lethality in which rate of cooking at one temperature is related to an equivalent time at a suitable reference temperature (usually 100 ºC) and can be used to determine the overall equivalent cooking time at the reference temperature for a changing process temperature.[27] The cook value is given by the equation:

$$C = \int_0^1 10^{\frac{T - T_{ref}}{z_c}} dt \tag{5.7}$$

where, Z_c is the *z*-value for cooking characteristics. The *C*-value at a reference temperature of 100 ºC (212 ºF) and a Z_c value of 33.1 ºC (91.5 ºF) is referred to as the C_0-value. The mass average value of cooking C_s, which is acceptable sterilization process[24] can be evaluated by the equation:

$$Cs = D_{ref} \log \frac{c}{c_o} \tag{5.8}$$

where, c_o and *c* are the concentration of heat vulnerable species at time = 0 and = *t,* respectively and D_{ref} is the *D*-value corresponding to the z_c-value.

During APP, the main goal of a food manufacturer is to obtain a commercially sterile product with minimum destruction of nutrients and an acceptable product with all the quality attributes. Therefore while selecting a proper time temperature combination; the food processors do consider the factors such as nutrient retention, and enzyme and microorganisms inactivation. *D* and z_c-values for destruction of nutritional and quality attributes are larger than that of the microorganisms. Typical *D* and *z* values for some of the heat-resistant spores are given in Table 5.1. The *z* value for microbial destruction is normally 10 °C while for the loss of desirable quality factors in the food such as nutrients it is 33 °C.[42] This signifies that rate of destruction of nutrients and sensory attributes at higher temperature is comparatively lower as compared to destruction of microorganisms and inactivation of enzymes. This forms the basis for aseptic processing of food materials and by using UHT sterilization commercial sterility with better retention of nutritional and quality attributes throughout shelf life can be achieved[15] To achieve commercial sterility factors which are to be considered are product characteristics such as pH, water activity, viscosity, composition, and dissolved oxygen.[27]

TABLE 5.1 Typical *D* and *z* Values for Some of the Heat-resistant Spores.[19]

Micro-organisms	z value (ºC)	D121 value (min)	Typical foods
Thermophilic (35–55 ºC)			
Bacillus stearothermophilus	9–10	3.0–4.0	Vegetables, milk
Mesophilic (10–40 ºC)			
B. cereus	36	3.8	Milk
B. coagulans	6–9	0.01–0.07	Milk
Bacillus subtilis	4.1–7.2	0.3–0.76	Milk products
Cl. botulinum toxins A and B	5.5	0.1–0.3	Low-acid foods
Clostridium sporogenes	8.8–11.1	0.7–1.5	Meats
Lactobacillus sp.	11.5–12.5	4.0–11.0	Tomato Juice
Psychrophilic (<5 ± 1.5 ºC)			
Cl. botulinum toxin E	10	3.0 (60 ºC)	Low-acid foods

5.4 ASEPTIC PROCESSING SYSTEMS

In order to achieve commercial sterility, the product must be heated to a given temperature for a specified period of time. Typical UHT processing

temperatures for low-acid foods (pH above 4.5) are 130–150 °C with holding times of a few, usually 4 s. Both indirect and direct heating systems are in use for processing of low-acid food products and high-acid (pH below 4.5) products such as juice are normally heated at 90–95 ºC for 15–30 s.[46] In UK, the minimum heat treatment for dairy products are set out in regulations (1 s at 135 ºC for milk, 2 s at 140 ºC for cream and milk-based products, and 2 s at 148.9 ºC for ice-cream mixes).[19] Steam or hot water is normally the source of heat for continuous thermal sterilization. Commercially sterile product can only be produced regularly if the product flow is constant, a sufficient overpressure in the system to avoid boiling and freeing of dissolved gases; and temperature of sterilization of the product must be controlled. There are two means of achieving sterilization temperature in a food production system: indirect heating system and direct heating system, which are described below:

5.4.1 INDIRECT HEATING SYSTEM

In the indirect system, the product and heating medium do not come in direct contact and is separated by stainless steel. Indirect heating modes include tubular, plate double-cone and scraped surface heat exchangers (SSHE), which are described below:

5.4.1.1 TUBULAR HEAT EXCHANGERS

Tubular heat exchanger employs either two or three concentric tubes as heat transfer surfaces. The tubes may be straight, spiral, or helical. Tubular heat exchanger consists of a pipe heat exchanger which is concentrically located inside another pipe. Tubular heat exchangers as compared to plate heat exchangers have thicker metal transfer barriers which make them capable to withstand higher internal pressures with less susceptibility to contamination. In food processing and dairy plants, triple tube heat exchangers (TTHEs) are typically used for milk and juice sterilization operating at temperatures up to 150 °C.[43] A TTHE consists of three concentric tubes which is an improved version of the double tube heat exchanger. In a TTHE, product travels through annular space and the heating medium flows through the inside of first tube and outside of middle tube. The main advantages of using TTHE over simpler tube heat exchanger include: larger area per unit length for heat transfer and increased overall heat transfer coefficients due

to higher fluid velocities in the annular regions. [47] The problem associated with a straight TTHE is that it occupies a large floor space. A helical triple tube heat exchanger is also an improved version of THE, which consists of three sections such as heating, holding, and cooling sections. In this sterilizer, milk flows in the middle annular portion of the heat exchanger and the heating media (steam) flow in the innermost tube and also in the outermost annulus. These heat exchangers are the most widely used ones for UHT sterilization. The tubular-based system are used for sterilization of fruit juices, white milk, flavored milk, cream, ice-cream mix, evaporated milk, desserts, and puddings.

5.4.1.2 PLATE HEAT EXCHANGERS

Plate heat exchangers are comprised of closely packed stainless steel plates, which are parallel to each other and pressed together in a frame. The stainless steel plates serves two purpose, one as to perform as a barrier and other to act as a heat transferring surface with product on one side and the heating medium on the other side. The number of plates can be adjusted to meet specific needs. Gaskets made of natural rubber or synthetic rubber seal the plate edges. Rapid heat transfer is guaranteed in plate heat exchangers due to the large surface and turbulent flow characteristics when compared to tubular heat exchangers.[8] David et al.[15] reported that plate heat exchangers are used primarily for preheating functions due to the difficulty in maintaining plate sterility. Production run time is limited more with plate heat exchangers than tubular heat exchangers due to fouling or burn-on. Factors which generally cause fouling are stability of milk proteins, temperature profile during heating, temperature differential between heating or cooling medium and product, presence of foam/gases, flow rate, and surface characteristics of the heat exchange surfaces. Plate heat exchangers have a number of disadvantages that are intrinsic in their design which include; lower flow rate and pressure tolerances, limitations with regard to the viscosity, high shear forces, shorter gasket life, higher risk for pinholes and stress cracking and relatively high load of heat is necessary. Burton[8] stated when plate heat exchangers are used in UHT processing they must be able to withstand greater temperatures and internal pressures and are generally used for milk, flavored milk, fermented milk, cream, coffee whiteners, and juices.

5.4.1.3 SCRAPED SURFACE HEAT EXCHANGERS

SSHE are used for product with high viscosity, concentrations up to 40% and for those having particles of up to 15 mm. SSHE is a jacketed cylinder in which the food product is heated with help of heating medium and consist of scraping blades which are mounted on a rotating shaft. The rotating action of the scraping blades within the jacketed cylinder serves two purposes that is prevention of fouling and improving the overall heat transfer. SSHE is usually deployed for highly viscous products due to lower energy efficiency and higher equipment cost as compared to the other indirect heating systems.[8] A wide range of products can be processed in SSHE like milk concentrate, yogurt, processed cheese, and whey protein concentrate products. Due to abrasive and excessive physical action of the scraping blades, food particle are damaged thereby affecting the rheological, textural, and sensory attributes.[10]

Indirect UHT systems are usually implemented for sterilizing of food products as they are relatively simpler in terms of operations, they can be easily dismantled for routine inspection, less requirement of floor-space, low installation cost, high regenerative energy recovery in case of plate heat exchangers may be up to 90–93%. As compared to plate heat exchangers, tubular systems have a lower heat transfer rate and running costs, but they have higher initial cost and more floor-space requirements. Tubular heat exchangers require limited service, are easy to operate.

5.4.2 DIRECT HEATING SYSTEMS

Direct heating systems consist of direct contact between steam (culinary) and product. Direct heating modes include steam injection (steam into product) and steam infusion (product into steam), which are as follows:

5.4.2.1 DIRECT STEAM INJECTION SYSTEM

In steam injection, steam is introduced into a pre-heated liquid product in form of fine bubbles using a steam injector which ultimately increases the temperature of the product to 150 ºC. After holding the product for a suitable time, the product is flash cooled to 70 ºC in a vacuum chamber which makes it possible to remove the condensed steam and volatiles. Pre-heating and final cooling take place in a plate heat exchanger or sometimes

in a tubular heat exchanger. Food grade steam must be used and it must not contain any incondensable gasses. Direct heating system has the advantage of rapid heating, which minimizes sensory quality changes in the product and reduces fouling problem and is found to be suitable for milk, cream, flavored milk, soya milk, and ice-cream mix. Relatively higher capital costs as compared to indirect systems and lower percentage of heat regeneration are the limitation associated with this method.

5.4.2.2 DIRECT STEAM INFUSION SYSTEMS

In this system, the product is pre-heated to ~75 °C in a pressurized stainless steel chamber in which the steam is infused using multiple nozzles. The product is heated until it attends a final temperature of about 142–146 ºC which is held for 3 s in a holding tube before flash cooling (65–70 ºC) in vacuum chamber. The degree of vacuum is adjusted to ensure removal of water added from the condensing steam. Due to exposure of product at very high temperature for a very short time, an outstanding product quality is obtained with desirable microbial destruction. Usage of steam infusion is advantageous over injection methods as the food product is not in direct contact with hotter surfaces and hence the chances of burning-on are minimized. Products treated by this system include milk and milk concentrate, cream, whipping cream, flavored cream, yogurt, soya milk products, baby food, ice-cream mix, processed cheese, puddings, desserts, and other very heat-sensitive products.

Advantages of injection/infusion heating systems are: lower total heat load which induces fewer chemical changes; less scaling and fouling rate which increases production runs; lower oxygen content in the product increases stability of vitamins and, during storage, reduces flavor changes caused by oxidation; and they are more suitable for viscous products. Whilst the disadvantages associated with the systems are: high initial and installation costs, low regenerative energy recovery (~50%), less effective against inactivation of enzymes and are technically complicated.

5.5 NOVEL THERMAL METHODS

Conventional methods of aseptic processing for particulate foods impair the organoleptic and nutritional quality, and cause mechanical damage to the particulates. Due to these adverse effects, different novel techniques of food

processing have been explored throughout the world to meet the increasing consumer demand for new and safe products with very high organoleptic and nutritional qualities for long-term use. Ohmic and microwave processing could be used for aseptic processing of dairy foods in near future.

5.5.1 OHMIC PROCESSING

Ohmic heating (OH) works on the principle of passage of alternating current (AC) through a food system, which serves as an electrical resistance and which causes generation of heat.[9] Most foods with water contents in excess of 30% are sufficiently good electrical conductors and can be heated by this method.[40] OH is a continuous high temperature, short time sterilization process in which the product to be sterilized usually passes over electrodes in one or more heating tubes followed by cooling in scraped surface, tube in shell or plate heat exchangers subsequently packaged aseptically.[16] Electrical energy is dissipated into heat, which results in rapid and uniform heating while maintaining the freshness, sensory, and nutritive properties of food products. The effectiveness of OH is affected by factors including intensity of electric field, time of exposure, temperature of food, and type of microorganism or enzyme. OH technology apart from economical and efficient it will help to provide consumers with minimally processed, microbiologically safe, and nutritious food products and hence considered as a promising technology for aseptic processing. Products such as fruit juices and concentrates, milk, puddings, soups, and liquid egg products can be heated rapidly, uniformly, and with a reduced impact on the organoleptic properties of the product. OH has been extensively used in the past for pasteurization of milk.[36] Heating rate of ~1 ºC s^{-1} can be achieved in liquid and solid phase.[27]

OH can be used for ultra high temperature (UHT) sterilization of foods, and especially those that contain large particles (up to 2.5 cm) that are difficult to sterilize by other means.[20] OH is reported to preserve food without significantly raising its temperature during processing, thus minimizing loss of vitamins and aroma, and change in color and flavor, as well as fouling and clogging of the equipment treatment zone.[37] The ability to heat particulate foods uniformly without mechanical damage, combined with lower nutrient and vitamin losses and no fouling of heat-transfer surfaces will ensure that OH will play a major role in the growing requirement for aseptic food products containing large particulates.[37]

5.5.2 MICROWAVE PROCESSING

Milk processed by direct or indirect UHT heating systems cause degradation of flavor and nutrients and to overcome these effects, an alternative method such as continuous flow microwave processing are explored to treat fluid milk products which can exhibit a longer shelf life. Several laboratory based continuous flow microwave heating systems are used for liquid foods with different configurations.[1] Microwave pasteurization of milk in a continuous flow unit has been tried by many researchers around the world. Hamid et al.[22] were the first group to use the technology for milk pasteurization. Instant start-up and rapid heating of food products make microwave heating suitable for aseptic processing of single/multiphase food products. In microwave sterilization system, the product is heated using microwave in the heating section up to sterilization temperature, held for a specified period of time in a holding tube, cooled in the cooling section, and finally packed aseptically in a sterile container. The process can be used for sterilization of foods especially with large particles (~ 2.5 cm)[19] Microwave heating involves rapid and direct heating of food product which reduces the exposure time to achieve the final temperature which ultimately reduces the total cumulative thermal treatment. Microwave heating treatment helps to preserve thermolabile constituents like aroma, vitamins, and pigments[34] and also maintaining the sterility by minimizing bacterial growth.[31]

Clare et al.[12] studied sensory attributes, microbiological quality, and biochemical parameters of microwave versus indirect UHT fluid skim milk during storage and advocated that microwave technology may provide a useful alternative processing method for extending the shelf life of aseptically packed milk products. The advantages of microwave heating as compared to conventional heating include: instant start-up; faster and selective heating; minimum fouling; improved food quality, energy efficiency, and control of the heating process. Disadvantage associated with microwave heating are non-uniform heating of the food materials. Due to cost, complexity, and non-uniform heating, microwave aseptic processing has not gained commercial acceptance. With the continued development this technology could be used in future for extended shelf life and aseptic processing of milk and milk products.

Regardless of the type of sterilizer used, the sterile product is cooled to 20 °C for low viscous food products like milk and fruit juices, and 40 °C for products of high viscous products like puddings and desserts. A pre-sterilized container is then filled with the cooled, sterile product. Aseptic surge tanks have been used in aseptic systems to hold the sterile product prior to

packaging. These tanks provide flexibility and act as a buffer between the APP system. A sterile air or sterile gas supply system is needed to maintain a positive pressure within the tank and to displace the content and in aseptic filling zone to prevent entry of contaminants.[19]

5.5.3 RESIDENCE TIME DISTRIBUTION (RTD)

The knowledge of residence time of fluid flowing in the different sections of the UHT sterilizer is very important. RTD is the time range between the entry point and exit of the fluid product form the holding tube.[44] The minimum residence time helps to ensure microbial safety of the product, while the average residence time is important in determining the quality of the food produced. Residence time distribution of fluid must be as narrow as possible to avoid heat induced changes like browning, loss of flavor and vitamins and obtain a uniformly heated product. Factors which govern the residence time are viscosity of the product, velocity, flow regime, fluid/solid relative density, particulate shape and size, pipe diameter and the particulate–liquid ratio.[2] For Newtonian fluids, the minimum residence time is half of the average residence time. Methods used to measure residence time distribution include direct observation with dyed particulates, time-of-flight, magnetic particulate sensors, and Hall effect sensors.[27]

5.6 PACKAGING FOR ASEPTIC PROCESSING

The aseptically processed products need to be packed in pre-sterilized container in aseptic filling zone. An overpressure of sterile air or other inert gas is induced and maintained in aseptic zone to facilitate the product removal and to deny any ingress from the environment. Some sterile and filling packaging systems (e.g., Tetra–Pak) use sterile air to remove excess hydrogen peroxide from the packaging material surfaces and to maintain a sterile condition in the filling zone. Sterilization of air may be achieved by heating the air and/or by passage through sterilizing filters.

5.6.1 SELECTION OF PACKAGING MATERIAL

The packaging materials for food products must provide containment, protection, preservation, and consumer information. The factors that must be

considered in selecting packaging material for aseptically processed foods are: permeation to oxygen, UV light and water vapor, mechanical properties, microbial barrier properties, stuffiness, durability, thermal stability, and sealing and chemical interactions. The package must be convenient, minimal in package weight, easy to handle and economic in the distribution chain, negligible injury risk when handling, safe for children, tamper safe, space saving in transport and storage; and the capital and operating cost must be minimal. Oxygen and light reduces the nutritive value, organoleptic quality, and shelf stability of foods. Hence, light and oxygen impermeable materials should be used. Aseptic package apart from protecting the product it also helps to guarantee the quality during storage; hence the structure as well as composition of packaging material varied depending on the product and package size and type. Generally more than one material in the structure of aseptic package is required to achieve required properties. The sealing strength must be adequate to maintain package integrity.[23] Package options for aseptic packaging include metal and rigid containers, web fed paperboard containers, preformed paperboard containers, preformed rigid/plastic containers, thermoform-fill seal containers, flexible bags/pouches, and blow molded plastic containers.[17] Examples of some packaging materials commonly used in aseptic packaging includes paperboard, aluminum foil, metalized film, stainless steel, polyethylene, nylon, polycarbonate, polypropylene, polystyrene, polyvinylidene chloride (PVDC), ethylene vinyl alcohol, and so forth. [15] Aseptic packages most commonly used are comprised of six different layers which are laminated and consist of paper (70%), plastic (24%), and aluminum (6%).

5.7 STERILIZATION OF ASEPTIC PACKAGING MATERIALS AND EQUIPMENT

In addition to sterilizing the product, an aseptic packaging system requires the sterilization of the packaging material and equipment before aseptic filling for successful production of aseptically packed products. Pre-sterilization of the parts of the packaging system through which the sterile packaging material or packages and the sterile product must pass prior to filling and sealing is a major source of post process contamination of UHRT treated products. Often the surfaces which are to be sterilized are massive metal parts like pipes and valves or sensitive forming devices for which elevated temperatures for sterilization are difficult to achieve.[8] Depending upon the construction and complexity of the aseptic filling equipment, sterilization

can either be done by using dry or wet heat alone; or a combination of heat and chemicals. Pre-production equipment sterilization is carried out using superheated water, saturated steam, superheated steam, or other appropriate treatments at an appropriate time/temperature sequence (e.g., 30 min at a surface temperature of at least 121 ºC) for commercial sterility.[25] Most commonly used method to sterilize the machine parts prior to aseptic packaging is usage of saturated steam as microorganisms are more resistant to dry heat, which necessitates higher temperatures.[8] The packaging material is sterilized by employing various sterilants such as: saturated steam, superheated steam, hot air, heating by extrusion process, ethylene oxide, chlorine, iodine, oxonia, food acids, ozone, hydrogen peroxide, light pulse, ultraviolet light, infrared radiation, gamma radiation, electron beam, and combination of these. Methods of sterilization of packaging materials have been discussed in more details by Ansari and Datta.[3] Hydrogen peroxide, hydrogen peroxide and UV light and, steam are widely used for decontamination of packaging film. The sterilization process employed should be established in terms of numbers of log cycle reductions of the most resistant organisms present on the food contact surfaces and the aim should be to achieve a minimum of four decimal reductions against bacterial spores.[30, 45]

5.8 ASEPTIC FILLING AND PACKAGING SYSTEMS

Aseptic packaging involves packaging of a sterile product into a sterile container under an aseptic environment followed by sealing the container hermetically so that sterility is maintained throughout the handling and distribution processes. An aseptic packaging system must perform the following functions:

a. Create and maintain a sterile environment in which the package and product can be brought together.
b. Sterilize the food product contact surface of the package.
c. Aseptically fill the sterile product into the sterilized package.
d. Produce hermetically sealed containers to prevent entry of spoilage organisms.
e. Record, monitor, and control critical factors.
f. Proper handling of the finished packed product to ensure container integrity.
g. Clean properly after use.

The processor must consider the sterilization of the product, the processing equipment, sterilization of the packaging materials and maintenance of sterile condition throughout the aseptic system. Temperatures reached and maintained during the sterilization process should be accurately determined by appropriate temperature measuring devices located at critical points in the system, or at least at the slowest heating (coldest) point. The APP system must be cleaned prior to equipment sterilization.

APP systems that are now in commercial operation in various countries in the world vary in design and degree of asepticity achieved. The commonly used two aseptic packaging systems fill UHT product either into preformed sterile packages or in form-fill-seal system.[14] Table 5.2 lists the general categories of aseptic filling and packaging systems based on the types of containers and the methods of sterilization of the containers. Aseptic packaging systems available for food product are mainly comprised of drum and bin systems, carton packaging machines, bag-in-box, bulk tanks and containers, plastic cups/pots/cartons, and pouches/sachets.[23]

TABLE 5.2 Different Types of Aseptic Filling and Packaging System.[15,17]

Systems	Package	Sterilants
Asepak	Bags	Heat
ASTEC	Bins and tanks	Pressurized steam
Combibloc	Cartons	Hydrogen peroxide + heat
Dole aseptic canning system	Steel/aluminum cans and lids	Superheated steam
DuPont Canada	Bags and pouches	Hydrogen peroxide
Evergreen	Cartons	Hydrogen peroxide + heat
Gasti	Cups	High pressure steam
Gaulin	Bags	Ethylene oxide
Hassia	Cups	Hydrogen peroxide + heat or pressurized steam
International Paper Co.	Rectangular packages	Hydrogen peroxide + heat
Liqui-Box Corp.	Bags	Gamma radiation
Metal-Box Fresh Fill	Cups	Hydrogen peroxide + heat
Pure Pak, Inc.	Cartons	Hydrogen peroxide + heat, oxonia
Remy	Cups	Hydrogen peroxide + heat

TABLE 5.2 *(Continued)*

Systems	Package	Sterilants
Remy	Bottles	Hydrogen peroxide or oxonia
Scholle Corporation	Bags	Gamma radiation or ethylene oxide
Serac	Bottles	Hydrogen peroxide
Tetra Pak, Inc.	Cartons	Hydrogen peroxide

The Dole canning system and Tetra Pak machines are the most common aseptic units used for packing UHT milk and milk products. The Dole aseptic canning system is being used in an increasingly wide range of commercial operations. Dairy products successfully packed with the Dole system include whole milk, evaporated and concentrated milks, flavored milks and other dairy drinks, milk-based baby food formula, cream, butter, and some types of cheese. Tetra Pak system is most widely used among the aseptic packaging systems in most of the countries of the world. In this system, packaging material is sterilized by dipping in hot hydrogen peroxide and filling chamber is sterilized by spraying with hydrogen peroxide. These systems are used primarily for dairy products and confections and the packaged products is shelf stable. The lower cost of flexible packaging materials compared with metal for can has stimulated increased interest in form fill seal aseptic packaging machine.

The different types of aseptic packages launched by Tetra Pak are Tetra Brik, Tetra Classic, Tetra Fino, Tetra Prisma, Tetra Rex, Tetra Top, and Tetra Wedge. Among these, Tetra Fino Aseptic, a carton-based pillow shaped package, is gaining popularity throughout the world due to good economy for producers as well as for consumers. It is a roll-fed packaging system based on the Tetra Brik Aseptic technology. Before any aseptic packaging system is approved for use, test must be conducted to: (a) bring the equipment to a condition of commercial sterility prior to production; (b) sterilize the air delivery system and produce sterile air; (c) sterilize the containers and lidding material; and (d) maintain sterility during production.

5.9 RAW MILK QUALITY

The quality of any finished product depends to a great extent on the quality of incoming raw material. Therefore, milk used for UHT processing must be

of very good quality.[21] Raw milk should be heat stable with low microbial counts and an acceptable flavor.[18] The raw milk most proffered for UHT treatment should have a milk fat (3.2–4.0%), pH (6.40–6.80), protein content (3.0–3.5%), standard plate counts (< 100,000 cfu/g for single producers and <300,000 cfu/g for commingled loads), titratable acidity (0.13–0.15%), and total solids (11.5–12.0%). Milk with increased acidity when subjected to UHT treatment, due to its poor thermal stability problems like sedimentation can occur, which drastically reduces the running time and also causes difficulties during cleaning operations.[17] Poor hygienic conditions, elevated storage temperature, longer storage of raw milk at refrigerated temperature are some of the common factors which affect the quality of the raw milk rendering it non-suitable for UHT treatment.

Milk stored at refrigerated conditions causes growth of psychrotrophic bacteria (*P. fluorescens*, *P. putida*, *P. fragi*, *P. putrefaciens*, *Acinetobacter* spp., *Achromobacter* spp., *Flavobacterium* spp., *Aeromonas* spp., and *Serratia marcescens*), which are able to produce extracellular enzymes that are extremely thermostable. The most important of these enzymes from the commercial viewpoint are the proteases and lipases, both of which are able to withstand high temperature short time and UHT treatments. Proteolysis of casein caused by plasmin is responsible for gelation and bitterness of UHT milk during storage. *Bacillus sporothermodurans* possesses high heat resistance, and results in milk discoloration and is a challenge to eliminate from contaminated processing equipment.[14]

The factors which influence the shelf life of UHT milk and which affects the gelation behavior are age of cow, stage of lactation, mastitis, season, microbiological quality of raw milk, storage temperature, fat content, and hydrolysis of lactose.[38] The detailed information is provided in a review on UHT milk processing and effect of plasmin activity on shelf life by Chavan et al.[11]

5.10 COMMERCIAL STERILITY TESTING

According to the WHO/FAO, commercial sterility of low-acid food is defined as follows "*Commercial sterility means the absence of microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during manufacture, distribution and storage*", according to Codex Alimentarius Commission (WHO/ FAO) CAC/RCP 40-1993. As per EU, "*UHT treatment is achieved by a treatment: (i) involving a continuous flow of heat at a high temperature for a short time (not less than 135 °C in combination with a suitable holding time) such*

that there are no viable microorganisms or spores capable of growing in the treated product when kept in an aseptic container at ambient temperature, and (ii) sufficient to ensure that the products remain microbiologically stable after incubating for 15 days at 30°C in closed containers or for seven days at 55°C in closed containers or after any method demonstrating that the appropriate heat treatment has been applied [Commission Regulation (EC) No 1662/2006 (amending Regulation (EC) No 853/2004)]."

UHT processes inactivate vegetative cells and spores of primary spore formers that is *Bacillus* and *Clostridium.*[39] Lewis[26] stated that for a longer stability microbial count of UHT milk should be less than 100 cfu/g even after 15 d of storage at 30 °C. Food and Drug Administrator (FDA) recommended an incubation and inspection program which can use a variety of standard and/or rapid methods for ensuring the commercial sterility. The incubation and inspection program should be representative for given lot codes, traceable through record keeping, and not a replacement for good manufacturing practices (GMP's).[2] Sampling plans are more extensive when commissioning aseptic filler than during routine production.[8]

A maximal acceptable defect rate of around 1 per 1000–10,000 samples is generally approved. In order to detect such defect rates, sampling statistics must be introduced which includes defining the level of probability (confidence level) to be used when checking for defect rates. Usually, a 90–95% confidence level of achieving a correct result is used. An ideal sampling plan provides sterility assurance within a reasonable cost structure.[18] Microbial testing should be viewed as an additional verification quality program and is completed through traditional and rapid methods.[17] Visual inspections, sensory analysis, and pH measurements are done in conjunction with rapid methods to verify product quality.

5.11 INTEGRITY TESTING OF ASEPTIC PACKAGES

Assessment of aseptic packages integrity is one of the most critical steps in the aseptic packaging of foods and the integrity of package must be maintained to ensure the safety and quality of the product during handling, distribution, and storage. Post process recontamination due to seal failure can result in reinfection of microorganisms. Before commercial production, a particular aseptic packaging system must be evaluated for the integrity of the aseptic package. To prevent problems related to loss of package integrity the processor must ensure to conduct a functional test of each new shipment; must always have a well conditioned and maintained equipment; well

skilled aseptic filling machine operator and supervisor; tertiary packaging and wrapping material must be of appropriate quality including the handling and transporting equipment to pack and handle aseptic packets; storage of the packaging material prior to use under suitable, hygienic conditions (temperature and humidity) and times. To evaluate and ensure the package integrity, the packets are subjected for visual inspection, dye penetration test or bubble testing, squeeze test, seal teardown, and conductivity. Additional tests identified by Holdsworth[23] include the inflation test, compression test, decompression test, biotesting, ultrasound imaging, mechanical tests, and headspace indicators. The mechanical tests on filled packages include stress testing, stack testing, load vibration, and impact resistance. Electrolytic testing of packages is being proposed to determine leakage through aseptic packages or damage to the inner layers of plastic. Plastic prohibits current flow unless the film has been damaged.

KEYWORDS

- **aseptic packaging**
- **commercial sterility**
- **functions of packaging material**
- **package integrity**
- **packaging material**
- **paperboard packet**
- **psychrotrophic bacteria**
- **UHT**

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CHAPTER 6

HIGH-PRESSURE PROCESSING OF DAIRY PRODUCTS

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CONTENTS

ABSTRACT

Consumers demand convenient, fresh like, nutritious products, and high pressure processing (HPP) is blend of all these preferences with no compromise in safety. It took 80 years to make its appearance in market but it has worldwide attention and recognition, as it is most promising technology. Soon in future, it can substitute the thermal technology and food with preservatives. Based on statistical research data, influence of high pressure and temperature to understand the fate of micro flora, need to investigate food items, in order to quantify processing parameters.

6.1 INTRODUCTION

Present consumers prefer minimally processed foods, which does not contain additives, are convenient to use and microbiologically safe. Although thermal processing technique is a well-established technique in guarding, the safety of food products, but it often degrades the nutritional value and sensory attributes. Thus, to ameliorate these flaws of thermal processing techniques (drying, freezing, pasteurization, and sterilization) on food, non-thermal techniques, such as irradiation, pulse electric field, high pressure processing (HPP), and mano-thermo-sonication are used as an alternative. HPP is a novel and promising technique, which largely encompasses the requirement of consumers and also overcomes constraints of thermal processing.[21]

HPP also known as "ultra-HPP", or "Pascalization" (named after Blaise Pascal) is a novel non-thermal food processing technique,[71] which involves the application of pressure (100–1000 MPa) that is instantaneously and uniformly transmitted throughout the food product using a transmitting medium (hydraulic fluid usually water). Transmission of pressure irrespective of food size and shape with small variation in temperature (2–3 MPa per 100 °C) depending upon composition of product.[10,26,27] The pressure of 1000 MPa generated using HPP is approximately 10 times equal to pressure at the bottom of deepest seas on the earth. The temperature can be controlled and varied between from 0 to 100 °C for a time period ranging from fraction of second upto 20 min, additionally HPP inactivates micro-organisms by using pressure under refrigerated condition. The unique features of HPP are:

a. Abilities to kill spoilage causing and pathogenic micro-organism and inactivate enzymes by pressure treatment eliminating use of heat or food additives.

b. Pressure transmitted is uniform and independent of product geometry (size, shape).
c. Helps in maintaining freshness, nutritional value, and qualities attributes of food.
d. HPP can be carried out at low temperature or at ambient conditions for heat sensitive products.
e. Time requirement for processing of food/food products is low.
f. HPP can be carried out in food products, which are with or without package.
g. HPP is also approved by regulatory agency in many countries and does have a positive consumer response.
h. Potential exists to change the functional and physical properties of food system and thus, can help in creating novel ingredients.[30,54,58]

6.2 HISTORY AND STATUS OF HPP

Blaise Pascal in seventeenth century studied effects of pressure on fluid to inactivate micro-organisms. Era of using HPP began at the end of nineteenth century with the pioneer work of Hite (1899) in milk.[22] He demonstrated effects of pressure at 600 MPa for 1 h at ambient temperature in reducing the microbial population with extended shelf life of milk (delayed souring). After that, ceaseless studies were carried out by other researchers emphasizing on the effects of pressure on different food products, such as egg albumen,[3] milk,[53] meat and its tenderization[47], and positive results were reported with regard to microbial safety. And pathogenic or spoilage causing bacteria, yeast, and mold responded differently to the pressure, which rely on parameters like processing time, temperature, and substrate, etc. High pressure induces conformational changes in cell membrane, cell morphology, and perturbs biochemical reactions as well as genetic mechanism, which ensure reduction in microbial count.

Although the research started long ago, yet, it was only after 80 years that attention was paid by Japanese manufactures in commercializing the technique considering the abilities of HPP to inactivate the microbial population and simultaneously maintaining the overall nutritive qualities of food. After overcoming the technical and packaging hurdles, it showed its appearance in the market (Meidi-ya Food Factory Co.) in 1990 for production of food items like jams, yoghurt and fruit toppings.[63] After its first commercial success in Japan, high pressure processed guacamole dip was introduced by Fresherized Foods, Texas, USA, followed by France (1995) and Spain (1997)

by commercializing orange juice, sliced cooked ham, tenderized meat, and oysters. During 2000–2007, a range of products including salsa, smoothies, meat, and poultry were launched into the market. Different model, manufacturers and research on different products are presented in Tables 6.1 and 6.2.

TABLE 6.1 Different Models of HPP Available in the Market.

Name of company	Model	Pressure-transmitting medium	Capacity/ inner diameter (L/mm)	Product [Ref.]
Stansted Fluid Power Systems	S-1L-100-250-09-W	Distilled water	2/100	Mango pulp, Shrimp[35]
				Black tiger[33]
				Litchi fruits[35]
				Black tiger shrimp[32]
	Pilot plant MINI FOOD-LABFPG5620,	70% ethanol–water solution mixed with castor oil used as lubricant	0.5	Pomegranate juice[17]
NC Hyperbaric Wave	6500/120, N.C.		120	Granny Smith apple purée product[44]
	6000/55	Distilled water	55	Olive jam[14]
Flow International Corporation, USA		Distilled water	2	Valencia and Navel orange juice[5]
		Distilled water	2	Australian navel orange juices[2]
		Distilled water	2	Vegetables (carrots, green beans and broccoli)[48]
		Distilled water	2	CumisMelon[70]
Resato International, Roden, Holland		Polyglycol	1.5	Navel orange juice[56]
				Reconstituted orange juice [55]
		Glycol–oil mixture (TR 15)	6-vessel (6 × 40 ml)	Tomato puree and strawberry juice[12]
		Distilled water		Carrot[15]
Avure Technologies Incorporated,		Distilled water	2	Pomegranate juice[62]

TABLE 6.1 *(Continued)*

Name of company	Model	Pressure-transmitting medium	Capacity/ inner diameter (L/mm)	Product [Ref.]
ABB Autoclave Systems, Inc., Columbus, OH)	Quintus Food Processing cold iso-static press model QFP-6		1.8	Strawberry based beverage[62]
NFM-Technologies		Distilled water		Strawberry[43]
ACB Pressure Systems	RCB300, San Francisco	Ethanol/water (50% v/v)	3.5	Ref.[6]
Hoefer Scientific Instruments		Distilled water	30 cm^3	Ref.[49]

TABLE 6.2 HPP and Key Findings: Fruit and Vegetable Products.

Product	Pathogens	Range (MPa/ min/°C)	Reduction (log cycles)	Optimum conditions (MPa/ min/°C)	Effect on qualities
Onion	Aerobic bacteria	100–400/30/25 and 40	4	300/30/40	Above 100 MPa induced browning and was strong even at 700 MPa due to damage in cell membranes resulted into release of poly-phenol-oxidase.[7]
	Yeast and mold		5		
Green beans	Total plate count	500/1/20	ND	500/1/20	Compared to conventional techniques there were more retention of ascorbic acid and firmness.[40]
Carrot, Spinach	*Salmonella typhimurium*	100–500/0–20/30	> 5	500/5/30	The color changes were noticeable in the cooked carrots and spinaches.[28]

TABLE 6.2 *(Continued)*

Product	Pathogens	Range (MPa/min/°C)	Reduction (log cycles)	Optimum conditions (MPa/min/°C)	Effect on qualities
Apple cubes	*Candida lipolytica, Escherichia coli*	200–650/10/25, 40	6	600/10/25	HP treatment had no measurable effect on the hardness of the apple pieces, but to prevent browning during the entire storage period, addition of sodium meta-bisulphite was necessary.[67]
Litchi fruits	Psychrotrophs	100–300/5–15/27	3.77	330/15/27	Extended shelf life by 32 days at refrigeration conditions (5 °C with minimal alterations in qualities attributes).[35]
Granny smith apple puree	Total aerobic mesophiles; Yeast and mold	400–600/5/20	<50	400/5/20	Total ascorbic acid and total phenolic content was not affected at 400 MPa but affected at 600 MPa and pasteurization.[44]
Mango pulp	Yeast and mold	100–600/0–20/30	4.6	600/5/30	Retained 85% ascorbic acid, 92% total phenolics, and 90% of antioxidant capacity of original mango pulp.[34, 35]
	Total *coliform*s		5.23		
	Lactobacillus		4.59		
Valerica juice	Total aerobic bacteria, yeast and mold, *Salmonella*	600/1/20	ND	600/1/20	There was no significant difference in qualities parameters like °Brix, viscosity, browning index and color, β-carotene concentrations, and ascorbic acid.[5]

TABLE 6.2 *(Continued)*

Product	Pathogens	Range (MPa/min/°C)	Reduction (log cycles)	Optimum conditions (MPa/min/°C)	Effect on qualities
Apple-Brocooli juice	*S. cerevisiae, Asper gillus-flavus, E. coli*	250–400/5–20	> 5 log	500/10/15	Nutritional substances (sulforaphane) in broccoli juice were preserved.[23]
Pomegranate juice	Total plate count	400–600/5–10/25–50	< 1	400/5/25	Treatment was effective to ensure complete elimination of microbial population with minimum alterations in qualities.[17]
Pomegranate juice	Aerobic mesophiles, molds and yeast	350–550/0.5–2.5/5	ND	350/2.5/5	Pressurization at 350 MPa phenolic content increased significantly between 3.38 and 11.99%.[66]
Olive jam	*Coliform*, *Bacillus cereus Salmonella and L. monocytogenes*	450–600/3–4	Below detection limit	600	Shelf life was similar to pasteurization but better sensory qualities were obtained with HPP jam.[14]

Avure and Hiperbaric are two major HPP equipment suppliers in the world, and they have retained most of the market share in western countries. On the other hand, Baotou KeFa, the Chinese market leader of HPP machine manufacturing, has partnered with US companies to expand into western markets. *Vision Gain* expects that the total number of industrial HPP machines installed worldwide will exceed 350 in 2015.

6.3 BASIC PRINCIPLES AND MECHANISM OF OPERATION

HPP mechanism is based on Le Chatelier's principle and isostatic principle (Fig. 6.1). Both principles[70] govern the dynamics of HPP processing.

Le Chatelier's principle states that increased pressure/physical compression leads to reduction in volume accompanied by conformational changes and phase transformation, and at constant temperature this increase in pressure enhances the degree of ordering of molecules of a given substance having an antagonistic effect on molecular structure as well on chemical reactions. **Isostatic principle** states that during compression, pressure is transmitted uniformly from all directions irrespective of the geometry of the product, thus the product as a whole retains its shape after decompression. This justifies the lethalities of HPP for microbial cells and yet maintaining the shape and texture of the food product.

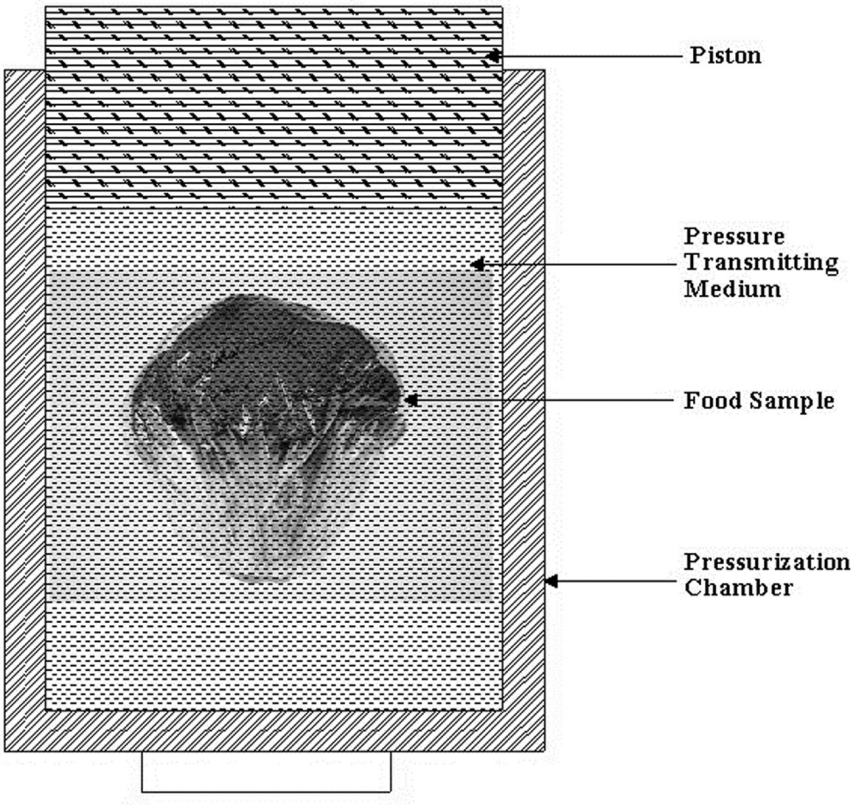

FIGURE 6.1 Pressurization of food using isostatic principle.

Packed food is kept into vessel, which is sealed from the top and bottom sides with fluid for transmitting pressure that is injected or supplied from a reservoir into vessel for displacement of air if any. After achieving desired pressure, pumping of fluid is stopped and maintained for the desired process time followed by decompressing and removal of packaged food product. The pressure transmitted throughout product is uniform, so it does not affect product geometry.

Flexible packaging material like ethylene vinyl alcohol and polyvinyl alcohol are most commonly used during HPP. Packaging material must withstand 80–90% compression of their original volume and regain their original volume after decompression.

6.4 EQUIPMENT DESCRIPTION

HPP used for processing of food is equipped with a pressure vessel known as heart of the equipment and closure valve, media for transmitting pressure, low-pressure pump to deliver hydraulic liquid, and thermostat to control temperature. Equipment can be utilized for batch, semi-continuous and continuous processing of fruits, vegetables, milk, poultry, fish, and meat products. Vessel size of HPP varies from 0.1 to 2 l for laboratory scale and for pilot scale the capacity ranges from 10 to 25 l and as high as to 300 to 400 l for commercial scale. Fluid used for transmitting pressure is mainly distilled water and on several occasions glycol-water solution, propylene with glycol, silicon oil, glycol–oil mixture, ethanol–water solution mixed with castor oil, polygycol inert gases, castor oil, and sodium benzoates are used as lubricants (Table 6.1).

Pressure transmission could be achieved using direct as well as indirect compression (Fig. 6.2)**.** In direct compression, pressure is applied on a fluid by a piston driven at the end of larger diameter using a low-pressure pump. It is a faster method of pressurization, but its use is limited to pilot scale or laboratory study due to restriction of high-pressure seal, which is placed between the piston and vessel's internal surface. In indirect compression, pressure transmitting medium is pumped from a reservoir into the vessel until the required pressure is achieved using a high-pressure intensifier.

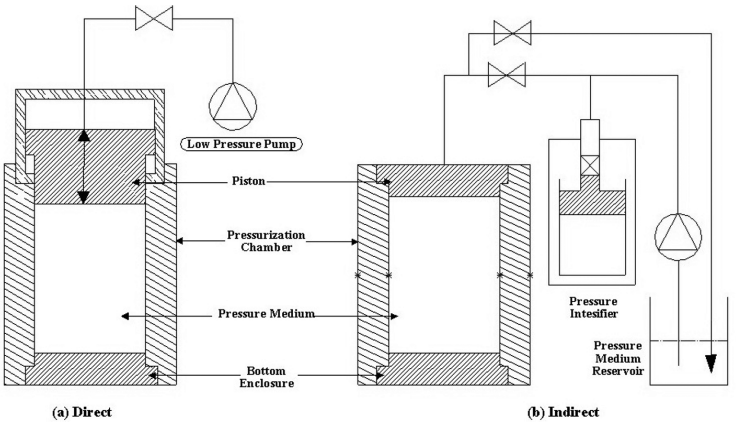

FIGURE 6.2 Schematic diagram for direct (left) and indirect generation of high-pressure (right).

6.5 MECHANISM OF MICROBIAL INACTIVATION

High pressure has a strong influence on the cellular architecture and functions. The lethal effect of HPP on microbial population is due to alteration in cell membrane permeability, changes in cell morphology, perturb biochemical reaction, interference in genetic mechanism, which occurs simultaneously in cell of microbes.[70] Factors governing the microbial inactivation are:

Intrinsic factors: nutrient content, pH, water activity;
Extrinsic factors: temperature, compression rate, and holding time; and
Factors associated with microorganisms: type, age, and morphology.

6.5.1 EFFECTS ON CELL MEMBRANE

Target for applying high pressure is to increase cell permeability and to alter or damage cell membrane. Physical perturbations include efflux of metal ions, leakage of adenosine triphosphate (ATP), and increase in uptake of dye, which leads to loss in functionality of cell membrane.[52] Fluorescent dye likes propidiumiodide normally does not penetrate the cell but when subjected to pressure, propidiumiodide penetration was observed in fluorescent micrographs. Induced changes in the cell envelope structure, often lead to loss of protein and RNA to the extracellular medium, which results into loss of membrane functionality.

Tholozan et al.[64] studied the effects of pressure (150–600 MPa/10 min) on *Listeria monocytogenes* strain Scott A and *Salmonella typhimurium* strain Mutton (ATCC13 311), and they reported that ATP content and intracellular were decreased with increase in pressure but the cell volume remained unaffected, lysis of cell occurred at a pressure of about 600 MPa.

6.5.2 EFFECTS ON CELL MORPHOLOGY

As compared to cell membrane, cell wall is less affected by high hydrostatic pressure. Using light microscope, no physical changes were prevalent in cell wall of prokaryotic and lower eukaryotes. But upon using scanning electron microscope (SEM), significant changes were observed on surface of cell with intracellular damage. Scars on outer cell surface of *Listeria monocytogens* and nodes on cell wall of *L. viridescens* were reported at 400 MPa using SEM by Ritz et al.[59] Kalchayan et al.[31] found alterations in cell wall and disruption of cell membrane integrity of *L. mesenteroides* when treated at 345 MPa (25 °C for 5 min).

6.5.3 EFFECTS ON BIOCHEMICAL REACTIONS

Another determining factor causing cell death is denaturation of the ATPase (key enzyme), reducing its synthesis in microfloradue to limited proton flow as an effect of high-pressure treatment. Effect of pressure treatment varies on enzyme depending upon its capability to withstand stress. Application of high-pressure retards reaction leading to increase in volume and favors reactions responsible for decrease in volume.[52] Upon application of high pressure among the primary, secondary, tertiary, and quaternary structure of proteins, primary structure is least affected, followed by secondary structure then tertiary structure. Irreversible denaturation occurs in primary structure at more than 700 MPa and at a 200 MPa the tertiary structure is affected significantly while quaternary structure is disrupted severely.[24]

6.5.4 EFFECTS ON GENETIC MECHANISM

Pressure disrupts the activity of enzymes engaged in DNA replication and transcription process of micro-organism leading to condensation of genetic material by degradation of chromosomal DNA hence affecting functionality

of nuclear material. Covalent and hydrogen bonds are least affected by pressure as compared to electrostatic and hydrophobic/ionic interactions. As hydrogen bonds are involved in formation of DNA helix structure, so nucleic acids are comparatively resistant to high pressure.[52]

6.5.5 EFFECTS OF HIGH PRESSURE ON FOOD QUALITIES AND SAFETY

HPP is already being used either at pilot scale or at laboratory scale to process fruits like apples, apricots, avocado, berries, cashew, apple, grapes, guava, mango, litchi, kiwi fruit, lemon, melons, passion fruit, peaches, pears, pineapple, and pomegranate. Apart from fruits, different fruit products including jam, juices, fruit salads, and others have also been processed by HPP.[57]

Kaushik et al.[35] treated litchi fruits at 300 MPa (10–15 min) and were able to extend the shelf life by 32 days at refrigeration conditions (5 °C) with minimal alterations in quality attributes including browning index and total soluble solids. Kaushik et al.[35] studied effect of HPP (100–600 MPa for 1 s to 20 min at 30 ± 2 °C) on mango pulp and change in biochemical, color, and microbiological properties. Best combination for reducing microflora with moderate variations in qualities for mango pulp was 600 MPa for 5 min.

Among the different vegetables for which research has been carried out using HPP are bitter-melon, broccoli, cabbage, carrots, cauliflower, garlic, ginger, green beans, mushrooms, onion, olives, spinach, potatoes, sweet potatoes, tomatoes, bell peppers, and red peppers.[57]

White cabbage treated at 500 MPa had a decreased soluble fiber but total fiber remained constant.[69] Li et al.[45] reported that microorganisms could be reduced to an extent below its detection limit by treating sour Chinese cabbage at 600 MPa. Kuo[42] showed that tomatoes treated at 200–500 MPa were able to retain color, extractable total carotenoids, lycopene, and antioxidant activity. The residual activities of pectin-methyl-esterase (PME) and poly-galacturonase (PG) were in the lower range of pressure (200 MPa for PME and 400 MPa PG, respectively), whereas these activities were higher for tomatoes treated at higher pressure (500 MPa).

Olive jam, when subjected to high pressure of 450–600 MPa and thermal treatment of 80 °C/20 min., had low degree of browning, more clarity, and high consumer acceptability. Whilst, treatment of olive jam at 600 MPa was sufficient enough to reduce *Salmonella* and *L. monocytogenes* below the detectable level, according to Delgado-Adamez et al.[14]

Apple-broccoli juice, treated at high pressure of 250–500 MPa/5–20 min, when compared with frozen juice, was comparable in terms of sensory and nutritional qualities upto 70 days of storage.[23] Significant inactivation (> 5 log cycles) of microbial load in fruit/vegetables juices can be achieved by treating at 500 MPa for 10 min and as a consequence the product may be free from yeast and mold, *coliform*, and salmonella up to 30 days when stored at refrigerated temperature (5 °C). Barba et al.[1] indicated that HPT (100 MPa, 120–540 s) of vegetable beverage containing tomato, green pepper, green celery, onion, carrot, lemon, and olive oil can retain more ascorbic acid as compared to thermal treatment (90–98 °C, 15–21 s), with lower changes in color profile and in total phenolic content.

Low pressure with less exposure time is effective in retaining phenolics, ascorbic acid, antioxidant, and anthocyanin in smoothies.[36,37,38] Smoothies treated with HPP (450–600 MPa/5–10 min/ 20 °C) when compared with thermally treated smoothies (70 °C ≥ 10 min), had high retention of antioxidant and total phenols.

Among the animal products (meat and poultry) and sea foods, products like pork (homogenates), pork paste, minced mackerel, fatty duck liver, low fat pastrami, Strasburg beef, Cajun beef, minced trout, oysters, chicken breast fillets, black shrimp, and chicken nuggets have been studied after subjecting it to different pressure and temperature combinations.

Noeckler et al.[50] studied the effect of pressure (50–250 MPa, 20–30 min at ambient temperature) on pork paste for keeping the *Trichinellaspiralis*as target organism. Initially, pressure of 50 MPa was not effective for inactivation, but at 150–200 MPa complete inactivation was achieved. Hayman et al.[20] employed HPP on ready-to-eat meat (low-fat pastrami, Strasburg beef, export sausage, and Cajun beef) at 600 MPa for 3 min at room temperature. After storage period of 98 days, microbial count of aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria* spp., *Staphylococci, Brochothrix-thermosphacta, Coliforms*, and fungi was well below detection limit and there was no significant differences in consumer acceptance of processed and unprocessed meat. Bulut et al.[51] reported that chicken meat treated at 400 MPa for 10 min at 0 °C had a total aerobic count well below detection limit.

Black tiger shrimp (*Penaeusmonodon*), when treated with high pressure processed (435 MPa for 5 min), achieved extended shelf life of 15 days and better microbial quality.[14] No significant changes were observed in Total volatile base nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N) levels of shrimp after processing. However, these were significantly increased with storage. Whiteness index was increased with pressure intensity imparting

brighter and mildly cooked appearance. Prawns (*Fenneropenaeusindicus*) treated with HPP (250 MPa/6 min/25 °C) can often destroy microorganisms including mesophilic, psychrotrophi, proteolytic bacteria, *Enterobacteriaceae, Pseudomonas spp*., H_2S producing bacteria, lactic acid bacteria, *Brochothrixthermosphacta,* and yeast and mold.[18] For oysters a reduction of 6 log cycles was achieved by Calci et al.[8] by treating it at 350–400 MPa/8.7–10.3 °C for 1 min. Based on the reported literature on meat, poultry, and sea food, it is advocated that HPP is a very effective technique for providing microbial safety with extend shelf life. HPP also helps to cause denaturation of proteins, which are responsible for holding the meat within the shell in oysters, mussels, crabs, and shrimp. Denaturation of proteins increases the yield of meat, which is separated from the shell; and also the operation becomes much easier and effective.

In dairy sector, HPP processed products include: milk and milk products and cheese like Mato, Gorgonzola, Cheddar, Gouda, and Camembert. Kolakowski et al.[39] achieved pressurized (400–600 MPa) milk with microbiological quality comparable to pasteurized (72 °C, 15) milk. Sierra et al.[60] also reported that HPP treatment of milk is a more gentle process rather than conventional methods to achieve extended shelf life. Goat milk processed at 500 MPa for 15 min has been stated to be as efficient as pasteurized milk.[4] Raw goat milk, treated at 400–600 MPa/7 min when used for cheese preparation, was effective in eliminating mesophilic aerobic bacteria, *Enterobacteriaceae,* lactic acid bacteria, and *Listeria spp.* The goat milk cheese, when compared for sensory attributes with untreated cheese, showed no significant differences.[13] Carminati et al.[9] studied effect of HPP on Gorgonzola cheese and reported 99% reduction of *L. monocytogenes* upon subjecting the cheese to a pressure of 600 MPa for 10 min or 700 MPa for 5 min, respectively. Low-fat yogurt, prepared from skim milk treated with combined treatments of high hydrostatic pressure (400–500 MPa) and thermal treatment (85 °C for 30 min), showed increased yield stress, resistance to normal penetration, elastic modulus, and reduced syneresis.[19]

From the findings reported by several scientists in this chapter, it can be concluded that HPP is a promising technique for reduction of microorganisms, which would give an extended shelf life without affecting the physio–chemical, textural, and sensory attributes. Key findings of application of HPP on different fruits, vegetables, meat, and seafood are summarized in Table 6.3 and for milk and milk products in Table 6.4. Some other applications of high-pressure processed product over, which studies has been carried out, are aloe-vera, green tea, rice wine, and honey.

TABLE 6.3 HPP and Key Findings: Meat and Sea Foods.

Product	Pathogens	Range (MPa/min/°C)	Reduction (log cycles)	Optimum conditions (MPa/min/°C)	Effect on qualities
Bovine muscle	Total microflora	50–600/0–5/10	2.5	520/5/10	Pressure higher than 300 MPa induces modifications of meat color parameters, such a decrease of the total color difference (DE), a decrease in the total flora, and a 1 week delay before microbial growth (520 MPa, 260 s).[11]
Ready to eat meat (low-fat pastrami, Strasburg beef, export sausage, and Cajun beef)	Aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria sp.*, *Staphylococci, Brochothrixthermosphacta, Coliforms*, and fungi	600/3/20	Below detection limit	600/3/20	Processed and unprocessed meat showed similar consumer acceptability (hedonic rating).[20]
Oysters	Total mesophilic count and anaerobic bacteria, H_2S producing bacteria	260–600/5/20	Note detected (ND)	400/5/20	HP-treated oysters showed significantly increased pH and lightness ($p < 0.05$) relative to untreated oysters; during storage, pH changed literstle in the pressurized oysters but decreased slightly in untreated oysters. Literstle changes in color were observed during storage at 2 °C on ice, compared to untreated oysters, which showed increased b-values (stronger yellow color). From tests of mechanical properties, HP-treated oysters showed significantly increased cutting strength ($p < 0.05$) with increasing treatment pressure compared to controls throughout storage.[11]

TABLE 6.3 *(Continued)*

Product	Pathogens	Range (MPa/min/°C)	Reduction (log cycles)	Optimum conditions (MPa/min/°C)	Effect on qualities
Chicken breast fillet	*E. coli KCTC 1682,* *Salmonella typhimurium KCTC 1925,* *L. monocytogenes KCTC 3569*	300–600/5/15	ND	600/5/15	The 300 MPa pressure significantly reduced flavor, aroma strength and juiciness, and 450 MPa produced breast fillets with the weakest aroma. Increasing pressure increased cooking loss and color by increasing L*, a*, and b* values. Moreover, elevated pressure increased hardness, cohesiveness, gumminess, and chewiness, as well as improved freshness of meat by reducing VBN. Pressure of 450 MPa and higher induced lipid oxidation.[41]
Beef	*E. coli O157:H7*	551/4/0–1	1.7		No significant effects on qualities attributes was observed except 4% decrease in cook yield.[29, 46]
Chicken nuggets	*Enterobacteriaceae*	300/5/27	3	300/5/27	No significant difference was observed in the color and textural properties of cooked and HPP treated chicken nuggets.[16]

TABLE 6.4 HPP and Key Findings: Milk and Milk Products.

Product	Pathogens	Range (MPa/min/°C)	Reduction (log cycles)	Optimum conditions (MPa/min/°C)	Effect on qualities
Milk	*L. monocytogenes;*	100–300/1–5 pulses/25	ND	300/3 pulses	Effective alternative to thermal technique to preserve milk and other liquid products.[65]
	E. coli O157:H7			200/3 pulses	
	S. enteritidis			200/5 pulses	
Milk	*S. aureus* ATCC 6538	400/21–31/ 0–50	6	400/ 30/21–31	Authors concluded that HPP might be a promising alternative to pasteurization of human milk. But require further research to evaluate the efficacy of HPP to inactivate relevant viral pathogens.[68]
	E. coli ATCC 25922		6		
	*S. aureus*ATCC25923		8		
	Listeria monocytogenes ATCC 19115		8		
Soy milk	*Enterobacteriaceae*	400–600/1–5/25 and 75	ND	600/1/75	HPP was effective to extend the stabilities of proteins in soymilk.[61]
Cheese slurries	*E. coli* K-12	50–800/ 20/10–30	ND	> 600/20/20 or > 400/30/20	Ref.[51]
	S. aureus ATCC 6538				
Gorgonzola cheese	*L. monocytogenes*	400–700 / 1–15	ND	600/10 or 700/5	Only one of the four pressurized cheeses was evaluated as different from the untreated sample.[9]

6.6 REGULATIONS FOR HPP

In European countries high pressure processed foods comes under categories of "Novel Foods and ingredients" (Regulation 258/97/EC) enforce since 1997. High pressure treated foods are considered novel because there consumption and production history so far is negligible and also they are produced by using new manufacturing techniques. According to "Novel Foods", legislation creates an evaluation and licensing system, which is mandatory for new foods and new processes.[25] No specific regulation is there for the high-pressure processed products. In USA to the high-pressure treated food traditional health regulation are applied. However, regulation needs to be enforced regarding robustness, qualities, and safety of the technology.

6.7 LIMITATIONS OF HPP

Compared to traditional processing thermal techniques, HPP is costly. The operating cost is almost same but the equipment cost is very high. But, previous statistics also showed that with increase in utilization of equipment and after its commercialization in many countries, capital cost is reducing. HPP is very effective in reducing vegetative and spoilage microorganisms except bacterial spores and enzymes being very resistant in nature, as they require elevated pressure to inactivate them. This hurdle can be sorted out by using temperature and pressure combinations. Also, almost food after HPP treatment requires refrigerated storages to retain food quality. Other limitation includes limited option available in packaging of food items. More study is required to explore effective packaging materials. To achieve antimicrobial effect, approximately 40% of free water should be present in foods to achieve the desired effect. Regulatory issues need to be resolved, before it is completely embraced by industries.

To overcome hurdle to inactivate most resistant spores, HPP when accompanied with thermal technique or other non-thermal technique can be resolved. Also with continuous effort and utilization of technique, equipment cost is reducing.

KEYWORDS

- **biochemical reaction**
- **cell membrane**
- **color**
- **fruits**
- **geometry**
- **high pressure processing**
- **inactivation**
- **isostatic**
- **isostatic principle**
- **Le Chatelier's principle**
- **lubricants**
- **meat**
- **micro-organism**
- **milk**
- **pathogens**
- **quality**
- **vegetables**

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CHAPTER 7

INTROSPECTION ON MECHANIZATION OF TRADITIONAL INDIAN DAIRY PRODUCTS

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CONTENTS

ABSTRACT

India is the largest milk producer in the world with about 140 million tons/year of milk. About 50–55% of this (i.e., 70–77 million tons) is diverted for preparation of Traditional Indian dairy products (TIDPs) at micro and small-scale level by local sweet makers. The TIDPs include: heat desiccated (*khoa* based), acid coagulated (*chhana* based), fermented (curd based), puddings preparation (with cereals), and frozen (with or by ice) products. These products are hereditarily inculcated in Indian life style. At present, the estimated market for these products is about $50 billion/year. In India, about 20–25% of total milk produced is processed by organized dairy industry for manufacturing of mainly fluid milk variants. Still, production of TIDPs is not taken up in big way by the dairy industry due to unavailability of quality milk, inappropriateness of variables and lack of standardization of process and absence of suitable packaging technology. Manufacture of TIDPs comprises of several unit operations and their respective equipments to finish these unit operations. In spite of above shortcomings, research institutions, entrepreneurs, and dairy industry have made significant inroads into mechanization and automation for production of TIDPs. It has been realized that there is a huge untapped potential ahead in future for these products. The organized sector has started production of *peda, paneer, mishit doi, gulabjamun, shrikhand, lassi*, and so forth, but on limited scale. For manufacturing of TIDPs, adoption of technology for more or less similar western product would be a feasible and economic option. This is the most opportune time to mechanize, so the TIDPs of uniform quality, balanced nutrition with hygienically packaging may be made available for the people of India and migrants of Indian origin throughout the world.

7.1 INTRODUCTION

Traditional Indian dairy products (TIDPs) have enjoyed a prominent position with their origin going back to the dawn of Indian civilization. They have been inculcated into life of every person in India and formed the indispensable part of festivals and ceremonies. TIDPs have played a significant role in the economic, religious, and nutritional wellbeing of Indian people since time immemorial. The “Operation Flood” one of the world’s largest and most successful integrated dairy development program, initiated in 1970, has led India to emerge as the largest milk producer in the world. It is estimated that milk production in India reached a record level of 140 million

tons in 2014 accounting for more than 17% of the world's total production. It is reported that about 50–55% of milk produced is converted by the traditional sector into variety of TIDPs.[6]

India has an enormously rich heritage of vast range of dairy products, which have an excellent potential for industrialization. The age-old art and craft of sweet-making needs to be transformed into an exact science and technology for developing appropriate equipment for the large-scale production. For attaining this objective, intensive innovative R&D and infrastructure are required. Faster growth may be achieved through integration with newly emerging, energy efficient unit operations utilizing advanced equipment for performing various unit operations required in the manufacturing process.[17] These initiatives will go a long way to develop dairy industry that meets the social, nutritional needs and conveniences for newly emerging life-styles. Tremendous scope exists for the strategic equipment development to improve the availability of value added dairy products for domestic market as well as exports. Figure 7.1 (a, b, c, d, and e) shows the rich heritage of Indian sweet cuisine in the form of heat desiccated, heat acid coagulated, fermented product, fat rich product, and milk-based puddings, respectively.

FIGURE 7.1 The rich heritage of Indian sweet cuisine.

The business provided by TIDPs and its accompanying value-addition, particularly in future, call for a thorough introspection of this sector. There is a need to look into various issues and accordingly re-evaluate to modernize traditional sector of dairy products.

This chapter presents the overall review on philosophy behind popularity, steps to be taken for, characteristics of recent innovations on adoption of western product making and development of equipment for TIDPs.

7.2 TYPICAL CHARACTERISTICS OF TRADITIONAL INDIAN DAIRY PRODUCTS (TIDP)

A variety of TIDPs are produced in India, some of which were region specific earlier, but have spread in other parts of country. Most of these products have distinguished sensory attributes and rheological characteristics with a particular chemical composition and microbiological quality. In India, many types of TIDPs are observed due to variation in preference, method of manufacture, sugar level, type of milk (cow, buffalo, or mixed), and so forth. A continued campaign is needed to determine the consumer's preference about the most desirable attribute in TIDPs. With this type of information only, organized dairies may exploit the untapped full potential of TIDPs.

The characteristics of several TIDPs are their mild cooked flavor which is developed through milk-metal-air contact during processing. It must be ensured that incorporation of this feature in equipment design. Since most of processing operations for TIDPs involve mixing and blending of various ingredients, the processing equipment must fulfill this requirement. The mechanisms are to be evolved with a high mixing index for a thoroughly blended homogenous product. Provision for visual inspection of the product during its processing would allow the correction in processing variable manually to achieve desired product quality.[26]

7.3 BENEFITS OF TRADITIONAL INDIAN DAIRY PRODUCTS OVER WESTERN DAIRY PRODUCTS

In India, deep-rooted tradition provided a considerable scope for diverting and channeling the considerable amount of milk for conversion into TIDPs. The major strength is the mass appeal enjoyed by such a wide variety of TIDPs. The market for these products far exceeds that for western dairy products. Their operating margins are also much higher, mainly due to lower raw material cost.[10] It is estimated that the raw material costs of *shrikhand, rasogolla, gulabjamun,* and *khoa*-based sweets (*peda, burfi, kalakand,* and *paneer*) is 29, 33, 34, 35, and 65% of the sale price, respectively. For western dairy products, comparative costs are relatively much higher varying from

70–80%. The production and marketing of TIDPs would give remarkable value addition to the extent of 200%, as compared to only 50% obtained by western dairy products. TIDPs can do wonders for organized dairy sector in terms of financial stability and future prospects. For TIDPs, Figure 7.2 (a, b, c, and d) shows the basis of evolution, causes of attraction, means of popularization, and their importance in daily life, respectively.

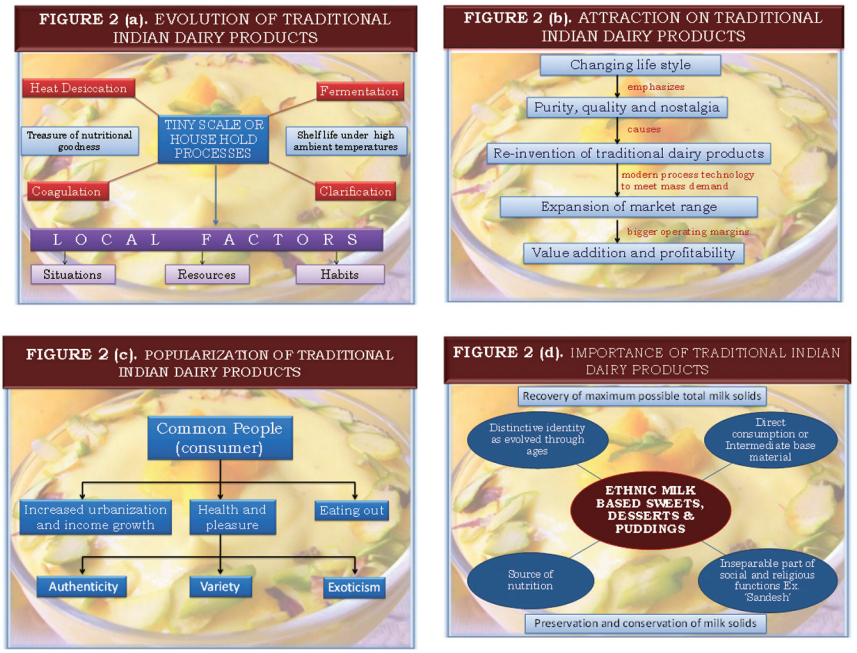


FIGURE 7.2 Traditional Indian dairy products**:** Basis of evolution, causes of attraction, means of popularization and their importance in daily life, respectively.

7.4 DRAWBACKS IN EXISTING SMALL SCALE PRODUCTION OF TRADITIONAL INDIAN DAIRY PRODUCTS

Manufacturing practices of milk products, which are adopted in most of the small sweetmeat outlets, are cumbersome, time consuming and labor intensive. Due to unhygienic conditions at the product site it is difficult to attain high microbiological quality standards. Consequently, chemical and organoleptic properties of TIDPs vary to a great extent. As most of TIDPs come under high moisture food, absence of cold storage facilities, lack of proper storage system and cold chain during distribution contribute to its

quality deterioration.[23] Its small-scale operations do not economically permit efficient utilization of fuel. Recipes for some of TIDPs are not standardized. Some variations were reported in chemical composition along with variations in sensory properties of TIDPs. The small-scale technology for the preparation of indigenous milk products cannot be exploited for industrial production.

7.5 SCOPE FOR MODERNIZATION OF SECTOR IN TRADITIONAL INDIAN DAIRY PRODUCTS

The typical demographic changes taking place in the world indicates increased requirement of processed, packed, and ready to eat convenient foods. India is no exception for this worldwide phenomenon. India's traditional dairy sector is poised for rapid expansion, hopefully with the application of modern technologies in production, packaging, and storage. The major strength of TIDPs is its popularity, taste, and texture. The margins of profit offered by TIDPs are lucrative and high because of low raw material costs (due to ingredient like sugar), least wastages (small scale permits close monitoring), remarkable value addition (up to 200% as discussed earlier) with minimum marketing efforts (due to goodwill, no advertisement needed) and least packaging cost (retailed in common available polyethylene or card board box).

7.6 SCIENTIFIC APPROACH FOR MANUFACTURING OF TRADITIONAL INDIAN DAIRY PRODUCTS

Characterization of various food products on the basis of their rheology and sensory evaluation forms the backbone of the scientific approach to product/process development. These parameters are also responsible for quality assurance in modern industrial practices. The current trend is to facilitate product description/specification for process control and thereby promote international trade. At this crucial period of time when the need for modernizing the manufacturing and marketing of TIDPs is being emphasized, such rheological studies would form a basis to obtain much needed information for product/process and equipment development.

In some research and development institutions, some studies have been directed toward rheology of selected indigenous dairy products such as *paneer, khoa, rasogolla,* and *sandesh*. It is also necessary to understand the

kinetics of texture formation during product preparation. Any equipment designed without taking into consideration of these basic aspects is less likely to be accepted by the people as the product obtained using such equipment would lack the desirable texture.

7.7 PRODUCTION OF TRADITIONAL INDIAN DAIRY PRODUCTS BY ORGANIZED SECTORS

In view of the growing awareness toward the hygiene, safety aspects of milk-based sweets in India, the consumer shall prefer to buy TIDPs from organized sector. Large-scale manufacture of TIDPs in a hygienically safe manner with assured quality control and proper packaging will certainly bring cheers for organized dairy sector. Dairy industry in western India is playing a lead role and has been followed by other dairies in various Indian states like Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, West Bengal, and Bihar. However, this production of TIDPs is much lesser as compared to total volume of variants of milk traded by any one of these milk processing plants.

A dairy plant in Baroda, Gujrat (India) manufactures *kesarpeda* by adopting a large-scale mechanized process which involves manufacture of *khoa* using continuous machine, heating *khoa*–sugar mixture in planetary mixer, cooling, mechanical forming of *peda* and packaging. Similarly, *gulabjamuns* are being manufactured commercially using *khoa* portioning and ball forming machines followed by deep fat frying and sugar syrup soaking lines.

7.8 EXPECTATIONS OF DAIRY INDUSTRY FROM RESEARCH INSTITUTIONS

Sometimes it has been claimed that Indian dairy industry is missing innovative processing technologies for traditional products suitable for large scale operations. However, successful efforts have been made to develop batch, semi-continuous and continuous equipment for *khoa, chhana, paneer, and ghee*-based sweets. In this direction, initiatives have to be taken by the industry for scaling up of processing equipment. Processes to be developed must reduce or completely eliminate the potential hazards commonly found in milk products.

7.9 ENSURING AVAILABILITY OF QUALITY OF TRADITIONAL INDIAN DAIRY PRODUCTS

Dairy industry has a responsibility to ensure the production and distribution of safe TIDPs. This effort will save consumers from health risks and diminish long-term impact of ill health in our economy. Today's buyers adopt "grab-and-go" convenience along with nutrition and health consciousness. Therefore, investment is essential to respond rapidly to customers who are increasingly demanding new and different taste experiences. The TIDPs containing health-promoting ingredients may be developed and promoted. Host of ingredients such as dietary fiber, cholesterol reducing phyto-sterols, minerals, and vitamins are available for value addition of TIDPs. Development of sugar free dietetic TIDPs is another area which eagerly waits for commercial exploitation.

One of the strategies to enhance international trade of TIDPs in world dairy market is to promote research by innovative techniques, designing of equipment and mechanization for the manufacture of value added dairy products.

7.10 WHY MECHANIZATION IS DESIRED?

Mechanization for manufacturing of TIDPs would give large-scale production with best possible hygienic and economical production. It would facilitate convenient handling of both raw material and finished products. The regular/scheduled inspection during all stages of processing would ensure control on product quality. By mechanization, it would be possible to use same equipment for its multiple utilization because most of processes in TIDPs preparation consist of same operations viz. heating, cooling, mixing, straining, pressing, and so forth with little bit variation in their level of process parameters.

7.11 PREJUDICE AGAINST MECHANIZATION

In spite of several innovative efforts made in the mechanization of manufacture of indigenous dairy products, adoption of these innovations by the industry is very limited. There may be several reasons for this. One reason may be typical mindset. There is always sense of reluctance in adoption of technologies developed locally.

Development of new prototypes of equipment for a wide variety of traditional milk products is a resource and time consuming exercise involving huge expenditure. Scaling up of successful prototypes may prove to be cumbersome. The equipment manufactures in India rarely invest in new equipment development unless they are assured of confirmed work orders from sizeable number of clients. Not much headway has, therefore, been made by equipment manufactures in adopting even the time-tested innovations reported by research institutes and the universities.

A decision regarding utilization of batch/continuous method is always debatable among dairy plant administrators. Because, even with immense popularity, they are not sure about possible TIDP's demand. Actually demand is so much that therefore they must realize it in a big way. Hence, at this moment, it will be appropriate to develop and promote batch type units so that mechanization of production in the small scale of unorganized sector may be started immediately. It would ultimately improve the hygienic quality of the TIDPs available in the local market.

The organized production does not necessarily mean large-scale production. Large number of tiny and small dairy product manufacturing units may be owned by or may have people with great innovative capabilities and basic skills. These talents need to the properly channelized in order to achieve long sited goal of innovative and hygienic production of TIDPs.

7.12 CONSTRAINTS FOR MECHANIZATION IN MANUFACTURING OF TRADITIONAL INDIAN DAIRY PRODUCTS

In spite of their large production and profit margin, knowledge base available on the technology of production, packaging, and preservation of most of TIDPs in India is rather limited.[21] Although in Indian market TIDPs have widespread popularity and acceptability even then organized sector has so far not been able to tap into this market potential for many reasons such as lack of published literature on their technology, inadequacy of appropriate technology for their commercial production, unawareness about appropriate packaging material, low keeping quality, and lack of quality assurance systems.

It is well known fact that the small-scale producers and tiny traders pay less attention toward food safety and standards in the preparation and distribution of TIDPs. Freshly prepared sweets are often packed in cardboard cartons to carry home purposes. Thus, cost of packaging and inventory is nearly saved by them. However, organized sector has to pack their products to enable it to keep safe for longer periods.

7.13 ADVANTAGES OF MECHANIZATION FOR PRODUCTION OF TRADITIONAL INDIAN DAIRY PRODUCTS

The mechanized production of value added dairy products will give the following advantages like (a) economic production, (b) uniform quality of the product, (c) hygienic production and better keeping quality, (d) scale-up production, (e) less laborious process, (f) less energy consumption, (g) better control over the process parameters to maintain standardized rheological and sensory attributes, and (h) promotes export of TIDP through small and medium entrepreneurs.

The mechanization for the manufacture of value added TIDPs will help in full exploitation of its commercial potential. It would also promote small entrepreneurship solely or through Public–Private Partnership (PPP) for the benefit of the society to get hygienic and best quality value added dairy products.

7.14 STRATEGIES FOR MECHANIZATION OF MANUFACTURING TRADITIONAL INDIAN DAIRY PRODUCTS

For mechanization, inducting most appropriate technologies for large-scale production is prime concern. The packaging is another field which demands utmost attention. By using modern packaging systems, shelf life of TIDPs can be extended for reasonable period of time. Identification and establishment of the regional preferences for the sensory and physico–chemical profile of the TIDPs must be completed as early as possible as reconnaissance work. Collecting and validating market intelligence for availability of raw materials, infrastructural facilities, market demand are essential to inspire prospective entrepreneurs to take commercial production. Scientific documentation of the package of practices for the production of traditional milk products must be made available in media including internet. Evaluation of newly developed processes/equipment must be done in relation to the products quality, shelf life, and consumer acceptability. Looking at the present awareness about food quality, it is also important to evolve a quality assurance system to meet the international standards of food hygiene and product safety. It would generate confidence in both domestic and international marketing circuits.

7.15 REASONS FOR PARTIAL MECHANIZATION FOR MANUFACTURING OF TRADITIONAL INDIAN DAIRY PRODUCTS

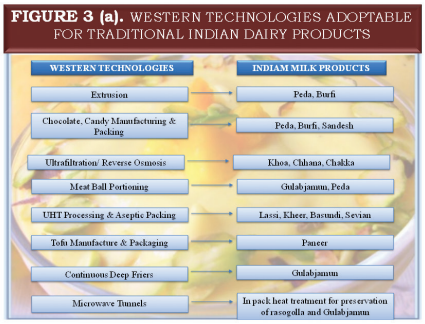
Prevailing Indian socio–economic fabric allows the changes to take place slowly but surely. With "White revolution" also, organized sector is processing only about 20–25% of total milk produced. The scientists and technologist did splendid work for understanding the logic behind various steps in preparation of various TIDPs. There is no dearth of equipment for completion of any unit operation involved in processing of TIDPs. The loose enforcement of food safety standards is ultimately discouraging mechanization. But now, time has come for reversal of this trend.

Sometimes inadequacy of appropriate technology, appropriate raw materials, packaging, and labeling for commercial production of TIDPs are cited as obstacles in mechanization. In stalwarts of dairy industry, ignorance about taking care of new pattern in consumer demand may prove to be detrimental factor toward mechanization. Generally, low keeping quality and lack of quality assurance system for TIDPs are other factors which adversely affect impending mechanization.

7.16 APPROACHES FOR MECHANIZATION FOR PRODUCTION OF TRADITIONAL INDIAN DAIRY PRODUCTS

It is always pertinent to note that designing and fabrication of altogether new equipment for a specific TIDP would prove beneficial to both processor (hygienic product) and consumer (low cost). The adaptation of available processing equipment for more or less similar food product (Indian or western) may be another way for manufacturing of TIDPs. The adoption of renewable energy source for full/partial reduction in fuel cost would create conducive atmosphere for mechanization among processors. Considering feasibility for mechanization and modern day preferences of common people, new dairy product can be developed and promoted. Figure 7.3 (a and b) shows two possible methods for mechanization of TIDPs namely adoption of technology for similar western dairy product[2] and basic components for new machine development, respectively.

Various investigators have designed and developed several equipment for mechanized production of value added TIDPs like *basundi, kulfi*mix, *kheer, khoa, peda, thabdi, burfi, gajarhalwa, dudhihalwa, halwasan,* and so forth with better hygienic, rheological qualities and improved shelf life at lower cost of processing.



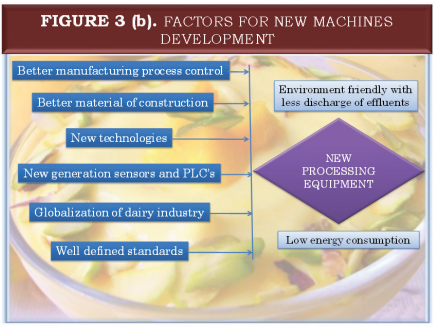


FIGURE 7.3 Two possible methods for mechanization of TIDPs namely adoption of technology for similar western dairy product[2] and basic components for new machine development, respectively.

7.17 FUTURE STRATEGIES IN DESIGN AND DEVELOPMENT OF NEW EQUIPMENTS FOR MECHANIZATION

In order to modernize the Indian milk sweets sector, it is necessary to understand the basic characteristics of TIDPs. The knowledge of these characteristics would contribute a great deal in design of equipment and standardizing scaled-up methods for manufacture of these products. There is also a need to facilitate formation of consortia of dairy industry to fund research to develop mechanized and energy efficient systems for manufacture and packaging.

With the rapid growth of dairy industry, the technology and design of process equipment has also undergone considerable changes and equipment for making indigenous products are no exception. The markets of conventional indigenous products are increasingly getting overcrowded and future success will depend on ability to provide innovative products, which consumers want. Whatever the innovation – products, processing method, or packaging – it should meet the real consumer need.

7.18 MECHANIZATION FOR MANUFACTURE OF TRADITIONAL DAIRY PRODUCTS

Increasing demand for TIDPs presents a great opportunity for the organized dairy industry to modernize and scale-up the production. In order to overcome the inherent disadvantages associated with conventional methods of manufacture of traditional dairy products such as inefficient use of energy,

poor hygiene and sanitation, non-uniform product quality, fatigue on the operator, and so forth; attempts have been made to develop batch, semi-continuous, and continuous equipment for the manufacture of these products. Figure 7.4 (a and b) shows the systems for production of *khoa*[15] and various Indian dairy products,[25] respectively. The brief review of some of investigations on mechanization of TIDPs is given as follows:

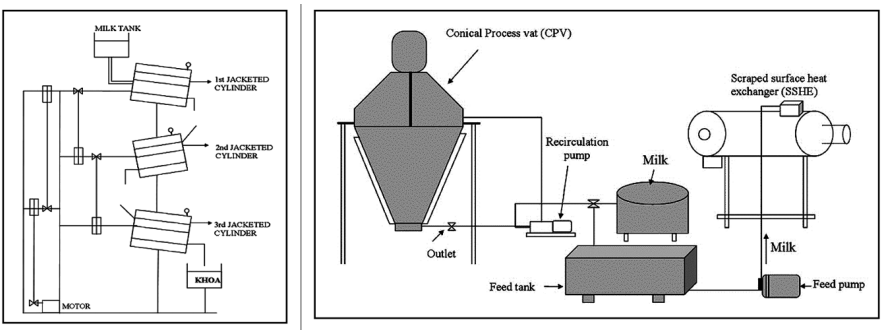


FIGURE 7.4 The systems for production of *khoa*.[15,25]

7.18.1 EQUIPMENT FOR KHOA AND KHOA-BASED TRADITIONAL INDIAN DAIRY PRODUCTS

In one of earlier investigations, attempts were made to develop semi-continuous *khoa*-making machine which was followed by batch type semi-mechanized SSHE, batch type mechanical conical process vat and scraped surface continuous *khoa*-making machines. The contherm-convap SSHE system is also being commercially used for the manufacture of *khoa*. Another TIDP, *burfi* in terms of better body and texture can be manufactured by integrating SSHE, conical process vat (CPV) and mechanized continuous cooling system.[27]

Successful attempts have been made to mechanize the methods of manufacture of *khoa*-based sweets like *basundi* and *halwasan.* A standard developed process for mechanized production of *basundi* using continuous machine by adopting standardized process and optimized parameters to have better rheological and sensory attributes.[20] There are various methods of *khoa* manufacturing machines: steam jacketed kettle/drum, conical vat, mechanized *khoa* pan, SSHE, and so forth. The thin film SSHE and inclined SSHE were found to have greater potential for industrial use.[15]

In order to compare the ability of SSHEs of different stages, uses of single stage thin film SSHE, two stage thin film SSHE and three stage thin

film SSHE have been used for *khoa* preparation. The advantages of three stage thin film SSHE over the others were uniform product quality, lower steam consumption, capable of handling both cow and buffalo milk.[5] A state of the art three stage SSHE has been developed with various instruments and process controllers.[7] This machine has been found very successful for large production of some TIDPs. The machine consists of three identical thin film SSHE. A feeding tank is provided with a screw pump. Change in speed of impeller provides variation in feeding rate, which is measured by magnetic flow meter. The steam inlets are provided with pressure gages, I/P converter, transmitter, pneumatic valves, air pressure indicator, and pressure controllers for automatic control. With variations in process and parameters, the machine was put in operation for continuous production of *khoa, basundi, rabri,* and *burfi*. The products prepared with best possible combination of operating parameters in SSHE had comparable characteristics with that prepared by standard batch process in proximate composition and physico–chemical characteristics. Three stage SSHE has also been used for manufacturing of *danedarkhoa* (variant for making TIDP called *kalakand,* etc.), which had all the textural parameters except hardness and adhesiveness as that of market sample having around same total solid content.[8]

In-line system for production of some TIDPs has been developed, which includes *khoa, burfi, rabri, basundi,* and *ghee*.[14,24] In this system, SSHE and CPV with agitator (fixed rpm) were integrated through suitable piping, pumps, and intermediate balance tank. These components were selected on the basis of unit operations, processing parameters, capacity, and suitability for integration into in-line system. Investigators found that the product obtained by the optimized parameters were comparable ($p < 0.05$) with products that were prepared conventionally. It can meet the requirements of small and medium entrepreneurs handling 500–2000 liters of milk per day for manufacture of these products with same set of equipment.

Continuous *basundi* making (CBM) machine has been developed, which consists of three SSHEs with specially designed scrapers and a chilling unit of two SSHEs, variable frequency drive (VFD) to facilitate variation in speed of scrapers.[19] The machine and standard process developed to manufacture *basundi* was found to have considerable industrial potential.

Many mechanized systems such as inclined scrapped surface heat exchanger, three stages thin film scrapped surface heat exchanger, convap–contherm, scraped surface falling film evaporator, and conical vat have been developed for *khoa* making. It has been claimed that some of these units are efficient in production of all varieties of *khoa* along with their products like *burfi, basundi,* and *rabri*.

7.18.2 EQUIPMENT FOR CHHANA AND CHHANA-BASED TRADITIONAL INDIAN DAIRY PRODUCTS

A prototype continuous *chhana* making machine has been developed involving tubular heat exchanger, acid injection chamber, holding coil, and strainer. In this machine, the sequence of operation consists of indirect heating of milk in a tubular heat exchanger to 95 °C, cooling to 70 °C, continuous coagulation with hot citric acid (70 °C) in a vertical tube, holding milk–acid mixture to permit complete coagulation, separation of whey in a continuous flow employing double wall basket centrifuge, and chilling to 4 °C by directly spraying chilled water on the layer of *chhana.*[4]

For production of milk acid coagulated products like *chhana, paneer,* and *sandesh* process parameters and machinery development have been mentioned.[4] *Paneer* was obtained by centrifugal pressing of buffalo milk curd. In *sandesh* making equipment, special arrangement was done for kneading and cooking of *chhana*–sugar mixture followed by dosing and shaping. Considerable research has been carried out for optimization of the process for the manufacture of *paneer*.

Developments have also been made in mechanization of *chhana*-based sweets. A screw conveyor has been designed for kneading of *chhana* and a cutter provided at the exit split the *chhana* into lumps of 10 g each. In this machine, the lumps were made to fall on a spinning disc and stationary disc above, which converts lumps of *chhana* into round balls.[4]

A kinematic half turn nut pressing mechanism has been designed for *paneer* in which a certain amount of angular displacement is provided on the half nut cylinder by pressure actuator for which there is simultaneous vertical downward movement of half turn nut cylinder to give correct amount of static pressure on the *paneer* coagulum.[11] In an investigation toward utilization of non-convertible energy sources, evaluation of performance of solar water heating system assisted surface heat exchanger has been conducted and its utilization for the manufacture of *paneer.* The surface heat exchanger's performance was evaluated by varying solar water flow rates. Various heat transfer parameters like log mean temperature difference, overall heat transfer coefficient, and so forth were evaluated for various combinations of hot solar water and cold milk through surface heat exchanger. The experimental *paneer* was compared with market sample for chemical, microbial and sensory characteristics which gave approximate equal value for both.[22]

Rasogolla is a delicacy cherished by Indian people throughout the year particularly in social and religious functions. In an effort toward its mechanization, *channa* kneader cum ball former has been developed. This

machine consists of feed hopper, kneading chamber, rotor, oscillator, reduction gearbox, and power transmission system.[28] The quality parameters of *rasogolla* prepared for mechanically kneaded and formed *chhana* such as percentage of absorbed sugar syrup, porosity, expressible syrup, and volume expansion were more or less at par with that of market samples.

7.18.3 EQUIPMENT FOR GHEE

Among all the fat rich dairy products, *ghee* occupies a prominent position. The various methods of *ghee* making viz. traditional method, creamery butter method, direct cream method, pre-stratification method, and continuous method have been discussed and compared. Comparison was made with the *ghee* attributes (percentage fat recovery, aroma, flavor, texture, aroma and flavor retention of storage, acceptability), equipment wise (no. of stages, clarification, and by product produced), and advantages/ disadvantages (domestic and industrial).[16] The creamery butter method was reported to be superior to others in most of parameters. In another set of experimentation, SSHE developed for continuous production of *khoa* was modified and with association of butter melter, has been used for mechanized production of *ghee* from butter.[13] The quality of *ghee* in terms of physico–chemical attributes and sensory properties indicated that *ghee* made from this process was comparable to that made with conventional method.

7.18.4 EQUIPMENT FOR MISCELLANEOUS TRADITIONAL INDIAN DAIRY PRODUCTS

Several investigations were carried out for mechanization of other milk products. Multipurpose twin cylinder thin film scraped surface heat exchanger was developed for manufacture of TIDPs like *basundi, kulfi mix, kheer, khoa, peda, thabdi, burfi, gajarhalwa,* and *dudhihalwa* with better hygienic, rheological qualities and improved shelf life at lower cost of processing.[20] Continuous *khoa, ghee, dahi,* and *lassi* making machine has been reported[18] and the mechanization for industrial production of *shrikhand, paneer,* and *chhana was stressed.*

Recently, development of in-line system consisting of milk tank, product conveying mechanism, CPV, and continuous product cooling system has been reported.[25] This in-line system is capable of producing various TIDPs viz. *khoa, burfi, basundi, rabri,* and *ghee*.

Development of process equipment for small-scale production suitable for home use (up to 20 L milk handling capacity) has been reported.[26] These were: (a) *paneer* making gadget, (b) mechanized *khoa* pan, (c) cream separator attachment (on home level mixture–grinder), (d) curd beater, (e) curd making machine, and (f) ice cream maker. Details about development of process equipment for medium scale entrepreneur/small dairy plant (between 200 and 500 L milk capacity) have also been reported. These were: (a) multipurpose vat for viscous dairy products, (b) batch type SSHE, (c) *chhana* making device, (d) continuous *chhana* ball forming and *rasogolla* unit, and (e) *rasogolla* cooker.

For preservation of milk fat, particularly in high temperature/relative humidity weather, *ghee* is the best option. By resorting to reverse engineering, the design for continuous manufacture of butter-G (product obtained by blending *ghee*, WPC, salt, and water) has been suggested.[3] The results had shown that it is possible to manufacture butter-G in a continuous manner by using the refrigerated conjugated intermeshing twin–screw system. It was also indicated that rheological properties of butter-G obtained at higher screw speeds were similar to the market butter sample.

The details about mechanization of production processes of some of TIDPs have been discussed.[1] Among these, thin SSHE for continuous production of *ghee* (with butter melter) and *khoa*, continuous *paneer* making unit employing twin apron conveyor system for dewatering, continuous *chhana* making machine, CPV for making multiple products, vented extruder for making *sandesh* and refrigerated extruder for converting *ghee* into butter, and so forth were prominent.

Many TIDPs have been developed in the past and some of these have cereals as an integral part. *Kheer* is one of them. A 100 kg/h continuous *kheer* making machine was developed.[12] The machine consisted of rice and sugar conveyors, a pressurized cooking section, a flash chamber, and a condenser. Relevant process parameters such as operating pressure and cooking time were optimized. Sensory trials of *kheer*, prepared conventionally and using pressurized methods were carried out.

Research has been conducted on native method for production of *makkhan* (a TIDP obtained by churning curd) by employing earthen pot (*matka*) and a wooden agitator (*mathani*).[9] Proper selection of combination was recommended to obtain higher recovery of fat with lesser churning effort and churning time. Figure 7.5 (a and b) shows arrangements of equipments for *chhana* and *gulabjamun* manufacture in their industrial production,

respectively.[2] Figure 7.5 (c) shows the system prototype for *paneer* production using solar energy to reduce fuel cost.[22]

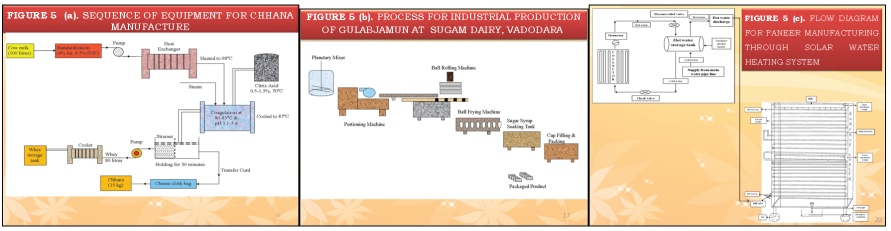


FIGURE 7.5 Arrangements of equipments for chhana (left), gulabjamun (center), and panner (right) manufacture.

7.19 SUITABILITY OF SCRAPED SURFACE HEAT EXCHANGER (SSHE) FOR TRADITIONAL INDIAN DAIRY PRODUCTS

For handling of high viscosity products with or without particulates, and for the products that tends to foul the heat transfer surface, the scraped surface heat exchanger (SSHE) is most suitable.[6] In SSHE, the working fluid is spread in the form of a film over the heat transfer surface by rotating blades. Each blade scoops a certain amount of fluid from the pool and accelerates it along the heat exchanger surface. At any given instant the fluid is picked up by the form of a fillet in front of blade. The blade action, which is similar to that of a plough, causes part of the fluid in the film to mix with that in the fillet. Simultaneously, re-storing of film thickness was obtained by allowing an equal amount of fluid to squeeze past the tip of the blade.

7.20 PACKAGING REQUIREMENT OF TRADITIONAL INDIAN DAIRY PRODUCTS

The traditional dairy products need to be processed and packaged in new form with guaranteed shelf life, chemical composition, and microbiological safety. Consumers always demand TIDPs and other milk foods with good flavor and texture. Statutory and regulatory provisions require that consumers receive foods of known standards and safety. Packages must be so intelligent that reduce or completely eliminate the potential hazards, which can develop during storage.

7.21 CONCLUSIONS

The TIDPs enjoy mass appeal, give high profit margins, and have high export potential. There is no alternative except modernization of this sector to produce high quality products with long shelf life. There is urgent need to generate basic data on these products to design new equipments or for efficient selection of processing and packaging lines. Industry, research and development institutions links need to be strengthened. Collaborative efforts of industry, equipment manufacture and research and development institutions are required for all round development of this sector.

With rapid growth of dairy industry, the technology and design of process equipment is also undergoing changes. The small-scale technology for the preparation of indigenous products cannot be exploited for industrial production; therefore mechanization of value added TIDPs is indispensable. Also, there is a need to develop innovative dairy products capable to permit mechanization during manufacturing. The value addition in milk (by converting into TIDPs) is an important aspect to be looked into by the dairy engineers, scientists, and technologists. The global dairy market would be open by introducing innovations in the designing of equipment for manufacture of safe and hygienic TIDPs. It would also help to have exciting opportunities for development of the rural economy because milk is produced there only.

KEYWORDS

- ***basundi***
- ***chhana***
- ***chhana*-based products**
- **conical process vat (CPV)**
- **continuous *basundi* making machine (CBM)**
- **dairy equipment**
- ***gulabjamun***
- **hygienic production**
- ***khoa***
- ***khoa*-based products**
- **mechanization**

- ***paneer***
- ***peda***
- ***rasogolla***
- **renewable**
- **energy**
- ***sandesh***
- **scraped surface heat exchanger (SSHE)**
- **thin film SSHE**
- **traditional Indian dairy products (TIDPS)**

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GLOSSARY

Basundi is a heat desiccated thickened milk dessert, having white to light caramel color, creamy consistency with soft textured flakes that are uniformly suspended throughout the product matrix.

Chhana is a product obtained from cow or buffalo milk or a combination of thereof by precipitation with sour milk, lactic acid, or citric acid. It should not contain more than 70% moisture and its milk fat content should not be less than 50% as the dry matter basis.

Ghee is a clarified butter fat obtained from cow or buffalo milk. It is produced by heat desiccation of *makkhan* (freshly churned butter)/butter cream at 105–110 °C. Heat changes milk proteins/lactose during the clarification process imparts a distinctive, pleased cooked flavor to ghee.

Kheer is a heat desiccated, cereal-based sweetened and concentrated milk confection.

Khoa is a product obtained by rapid drying of milk or a combination of thereof. The milk fat content shall not be less than 20% of the finished product.

Paneer is obtained through heat/acid coagulation of the casein component of standardized milk, entrapping through complex physico–chemical interactions all the fat and part of denatured whey proteins and colloidal salts as well as a part of the soluble milk solids.

CHAPTER 8

STANDARDIZATION OF HEAT-DESSICATED PRODUCT: MILK CREAM

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CONTENTS

ABSTRACT

The name *khurchan* means leftover scrapes, in Hindi. Like other traditional milk products, *khurchan* is mostly produced manually on cottage scale with variable quality. Boil the milk, scrape off the leftover from the *karahi* bottom, slap it on a tray, sprinkle *bhoora* (powdered sugar), cut into pieces, and serve. No systematic work has yet been carried out in respect of its quality. In view of the rapid growth of organized dairying in India, with greater emphasis on the production of indigenous dairy products an attempt has been made to standardize the procedure of manufacturing and to attain uniform quality. Buffalo milk was collected from local market and standardize for 6% fat and 9% solids not fat (SNF). Good quality sugar was purchased from local market. Shallow pans (*karahi*) of various thicknesses and diameter were purchased from market providing requisite thickness and diameter on order basis. Iron shallow pans were used for preparation of *khurchan* having diameter of 8 cm with varying thickness of 10, 15, 20, and 25 mm. Standard whole milk (one liter at a time) is kept on an open fire in a shallow pan (*karahi*). Evaporation was done without stirring. During evaporation clotted cream (m*alai*) was removed and kept at colder side of pan. Quality of milk and thickness of pan have remarkable impact on the quality of product. It has been found that most acceptable product involves standardization of buffalo milk for 6% fat and 9% SNF and iron shallow pan (*karahi*) of 20 mm thickness and this product scored high 8.5 on 9-point hedonic scale. The chemical composition of product was also determined. It had 22% moisture, 25% fat, 17% protein, 15% lactose, 18% sucrose, and 3% ash. It was observed that thickness of the pan has remarkable impact on the quality of *khurchan* due to heat induced interactions of its constituents.

8.1 INTRODUCTION

Dairying in India is a main source of livelihood for majority of rural households. The dairy industry is the major growth engine in many areas of the country, being a major source of rural income apart from improving nutrition and increasing women empowerment. Market specialists predict that the Indian dairy industry is going to double in the next five years. This gives us opportunities for investment, marketing, exporting, value addition, and employment generation. However, with every opportunity, there are challenges too.

India continues to be the largest producer of milk. The share of India in world milk production has been steadily growing. The growth rate of milk

production over the last 10 years has been over 4.0%. During the last five years, India has been adding around 5 million tons of milk, which is single largest agricultural commodity in India leaving behind wheat and rice. India is also the largest market for milk and milk production and per capita milk availability is about 302 g/day.

Demand for milk and milk products has been rising at faster rates on account of increase in GDP, increase in rural areas, changing food purchase basket to informed consumers. Successive rounds of consumer expenditure survey of National Sample Survey Organisation (NSSO) indicate that more people now consume milk both in urban and rural areas. Milk consumption incidence has increased from 62% in 1987–88 to 78% in 2011–12 in rural areas; and in urban areas it has increased from 79 to 85% for the same period.

The food habits in India are changing. Consumers now demand more value-added products like cheese, ice cream, probiotics, yoghurt, nutraceutical, and milk based personal care products. Given rising income of the consumers and changes in the familial relationship, demand for such products has been on the rise, creating increased opportunities for the industry.

Until recently, India has, by and large, been able to meet the domestic demand without resorting to any significant imports. However, despite the large production, only about 15% of the total milk production is handled by the organized sector. There lies a great challenge and opportunity both in the unorganized trade and consumption. Out of total milk production in the country, only 30% is processed by the organized sector.

There is ample scope for product diversification. About 43% of the milk produced in the country is sold as liquid milk and of the remaining 55% of milk, about 35% goes in ghee and butter. Hence, there lies a great opportunity in increasing our product. At present, dairy industry has almost negligible amount of value-added products.

Khurchan is an indigenous concentrated, sweetened whole milk product. *Khurchan* is believed to have its origin in Madhya Pradesh, India**.** It is quite popular in northern parts of India. However, very little scientific data and published information are available on this sweet except for the laboratory made *khurchan* and quality characteristics of *khurchan* marketed in Chhattarpur town of Madhya Pradesh.[5]

The traditional milk sweets and desserts prepared by heat desiccation and by subsequently adding sugar have great relevance to Indian culture such as *peda*, *burfi*, *basundi*, *Kheer*, etc. *Khurchan* is produced manually on cottage scale with variable quality depending on the skills of *Halwais* (sweet makers).

Khurchan is used for direct consumption. It is produced in the Northern region of India almost exclusively from buffalo milk as it gives a higher yield

than the cow milk. The final produce has a slightly cooked flavor, which is relished. It contains all the milk solids in an approximately five-fold concentration, together with an addition of sugar, so its food and nutritive value is very high. Typically body of *khurchan* consists of firm layers of milk solids. It has a rich, smooth but somewhat chewy texture. This product has a distinctive pleasant caramel/cooked flavor. Heat-clotted fraction of milk absorbs unclotted milk in the liquid phase, which imparts softness to the product.

The preparation is simple: boil the milk, scrape off the leftover from the *karahi* (iron pan) bottom, spread it on a tray, sprinkle *bhoora* (powdered sugar), and serve. There is a need to standardize the method of manufacture incorporating principle of energy conservation and for attaining uniformity in quality. No systematic work has yet been carried out in respect of its quality. In view of the rapid growth of organized dairying in India, with greater emphasis on the production of indigenous dairy products, an attempt has been made to standardize the procedure of manufacturing and to attain uniform quality.

The 9-point hedonic scale (also known as degree-of-liking scale) is the most common hedonic scale for measuring product liking by consumers.[1, 3, 6] Differently coded samples are presented to consumer panelists one at a time and they are asked to rate their hedonic response on the scale that can be in a vertical or horizontal line without affecting results. The scale (Fig. 8.1) is based on equal interval, which is important in the assignment of numerical values to the response choices (from 1 = "dislike extremely" to 9 = "like extremely") and to the use of parametric statistics in analysis of the data.

This chapter presents standardization of heat desiccated milk product: milk cream (*khurchan*).

8.2 METHODS AND MATERIALS

Buffalo milk was collected from local market and standardized for 6% fat and 9% solids not fat (SNF). Good quality sugar was purchased from local market in Chhattarpur, Madhya Pradesh, India. Shallow iron pans (*karahi*) of various thickness and diameter were purchased from local market providing requisite thickness and diameter on order basis. Following types of iron shallow pans were used for preparation of *khurchan*:

1. Diameter 8 cm, thickness 10 mm = T1.
2. Diameter 8 cm, thickness 15 mm = T2.
3. Diameter 8 cm, thickness 20 mm = T3.
4. Diameter 8 cm, thickness 25 mm = T4.

8.2.1 MANUFACTURING METHOD FOR KHURCHAN: STANDARDIZATION OF PROPERTIES

Standard whole milk (500–700 ml at a time) was kept on an open fire in a shallow pan (*Karahi*) having thickness of different sizes. Evaporation was allowed without stirring. During evaporation, clotted cream (*malai*) was removed and kept at colder side of pan. Almost 80% of water was allowed to evaporate.

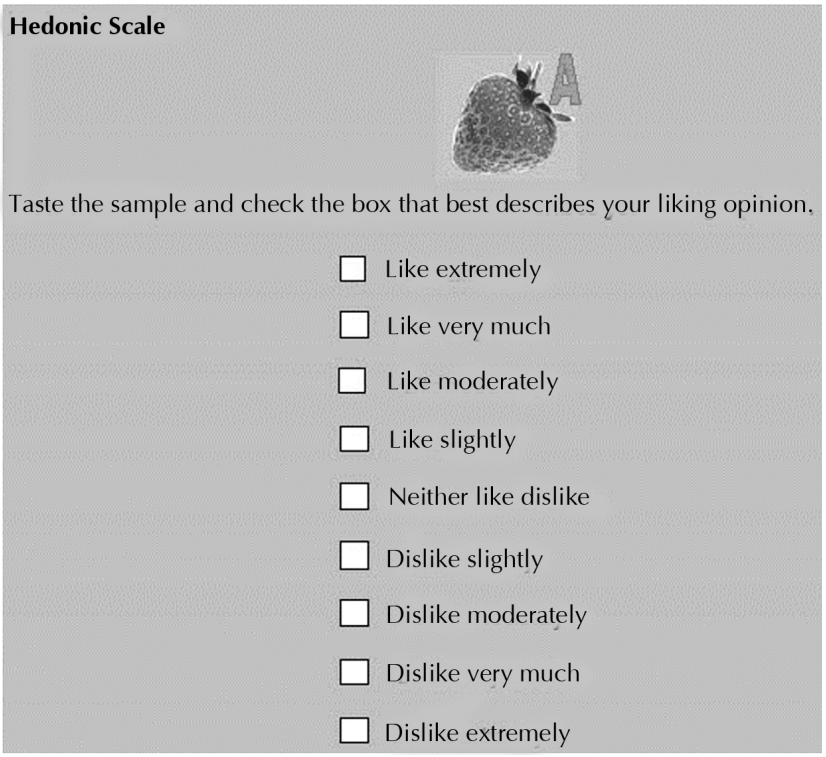

FIGURE 8.1 Hedonic scale.

8.2.2 SENSORY EVALUATION

Samples (T1, T2, T3, and T4) were subjected to sensory evaluation using 9-point hedonic scale, scored by the panel of five judges (BIS) (Fig. 8.1).[2] The scores obtained by the different samples on the different sensory characteristics are presented in Table 8.1. Data presented in table are the average

of three replications. The evaluated sensory characteristics were color and appearance, texture, mouth feel, and overall acceptability of the samples.[5]

8.2.3 CHEMICAL ANALYSIS

Samples of *khurchan* (Fig. 8.2) were analyzed for chemical analysis. A representative sample was passed through a fine mash stainless steel grater and was ground using an electrical grinder. The ground product was placed in a sample container and was used for chemical analysis.

FIGURE 8.2 Preparation and samples of *khurchan*.

The moisture and total solids were determined by gravimetric method (ISI)[4] with minor modifications, which involve addition of 5 ml of hot distilled water to disperse the solid matter uniformly. Fat content was estimated by the Gerber method. The protein content was determined by the Kjeldahl method. Lactose and sucrose were determined by Lane and Eynon method.[4] Ash content was determined by the standard method.[4] The results pertaining to the major milk constituents are given in Table 8.2.

8.3 RESULTS AND DISCUSSION

Khurchan is mostly produced manually on cottage scale with wide variation in its quality depending on the skills of *Halwais* (sweet makers). Quality of milk and thickness of pan have remarkable impact on the quality of product, therefore an attempt was made to standardize the procedure of manufacturing to attain uniform quality.

It was found that most acceptable product involves standardization of buffalo milk 6% fat and 9% SNF and iron shallow pan (*karahi*) of 20 mm thickness. This product scored 8.5 for each of the sensory attributes on 9-point hedonic scale (Table 8.1).

The chemical composition of product was 22% moisture, 25% fat, 17% protein, 15% lactose, 18% sucrose, and 3% ash (Table 8.2). It was also observed that thickness of the pan had a remarkable impact on the quality of *khurchan* due to heat induced interactions of its constituents.

TABLE 8.1 Effect of Thickness of Pan on Sensory Characteristics of *Khurchan*.

Thickness of pan, mm	Color and appearance	Texture	Mouth feel	Overall acceptability
10, T1	8.2	7.5	7.6	7.7
15, T2	8.2	8.0	8.0	8.0
20, T3	8.4	8.5	8.5	8.5
25, T4	8.2	7.6	7.8	7.8

TABLE 8.2 Chemical Composition of *Khurchan*.

Parameter	Units	T1	T2	T3	T4
Ash	%	03	03	03	03
Fat	%	22.6	23.6	25	22.6
Lactose	%	14.2	14.8	15	14.1
Moisture	%	27	25	22	27.2
Protein	%	14.6	15.6	17	14.6
Sucrose	%	18.6	18.0	18	18.5
Total solids.	%	73	75	78	72.8

8.3.1 TECHNOLOGY

Manufacturing of *khurchan* is a highly skilled technique and requires considerable experience and constant attention. Buffalo milk is preferred for its richness and the quality. In batch operation, a small quantity of milk, usually 500–700 ml is simmered over slow fire in a shallow pan (*Karahi*). Milk is allowed to concentrate without excessive stirring. Temperature, at which evaporation is done, affects the color of the finished product. Quicker evaporation at higher temperatures yields a whiter end-product, while slower evaporation at lower temperature makes the end-product somewhat brownish in color. When almost 80% water is evaporated, pan is allowed to cool. After cooling, thin layers of clotted milk are formed and are cut into layers with the help of khunti and are scraped. Its remaining portion of concentrated liquid is poured over the solid layer. Then, powdered sugar (5% by weight of original milk) is sprinkled over it, which quickly dissolves into the liquid fraction of concentrated milk.

8.4 CONCLUSIONS

Most of the dairy sector in India has been solely dependent upon sale of liquid milk, milk powder, and ghee. This has made the sector work on very thin margins. Till recently, there had been negligible growth of value-added dairy products. In view of the maturity that the dairy is now approaching fuelled by consumer demands, it is important to get high-margin value-added products. If the domestic sector is unable to deliver the same, the space would be occupied by imported value-added dairy products. In view of the rapid

growth of organized dairying in India, with greater emphasis on the production of indigenous products, an attempt has been made to standardize the procedure of manufacturing and to attain uniform quality, because *khurchan* is dying soon and it is difficult to find this sweet. It is not widely available. Overall, the dairy industry is undergoing metamorphosis. The industry is still at its early stages and would need several phases of growth.

KEYWORDS

- ***bhoora***
- **buffalo milk**
- **Bureau of Indian Standards (BIS)**
- **clotted cream (*malai*)**
- **evaporation**
- **fat**
- **hedonic scale**
- ***Indian Dairyman***
- **iron shallow pan**
- ***ISI***
- ***khurchan* (leftover scrapes or milk cream)**
- **lactose**
- **milk**
- **milk products**
- **protein**
- **SNF**
- **standardization**
- **sucrose**
- **hedonic scale**

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CHAPTER 9

HEAT-DESSICATED MILK PRODUCT: A CONDENSED MILK

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CONTENTS

ABSTRACT

It has been observed that the traditional Indian dairy products are by and large produced by small and medium milk sweet makers in the urban areas with exception of few large manufacturers. Traditional methods are usually used by the sweet meat makers which affect their keeping quality and taste. Mechanization of *khoa* and *khoa* based sweets manufacturing has enabled the dairy plants to produce the sweets on a commercial scale under more hygienic conditions thereby increasing the shelf life and consumer acceptance of the product. Keeping in view the demand of the traditional Indian dairy products, a concentrated effort has to be made to improve the keeping quality of products by using appropriate process technology, suitable packaging materials, storage, transportation, and marketing for long sustainability and profitability.

9.1 INTRODUCTION

India is emerged and retained its status as the largest milk producer in the world since last 15 years. Milk is highly perishable commodity. In order to preserve this valuable source of nutrients, more than half of milk produced in India is converted into a variety of traditional value added milk products. These products play a significant role in the Indian economy. *Khoa* is a heat desiccated value added indigenous milk product. Due to its large scale consumption, about 9.15×10^5 tons of *khoa* is being manufactured annually, which is equivalent to 7% of India's total milk production.

This chapter presents brief overviews on traditional method of *khoa* making, technological up-gradation in *khoa* making process, its shelf life, *khoa*-based sweets, and storage of *khoa*. *Khoa* is an ethnic Indian condensed milk that is prepared by heat desiccation of fresh milk [http://www.shabdkosh.com/translate/condensed%20milk/condensed%20milk-meaning-in-Hindi-English].

Milk is used as an important food commodity since ancient times in Indian subcontinent. It plays a vital role in the diet. India's milk production is 133.7 million metric tons during 2013–2014. In India, the share of milk and its products is the largest after cereals, and it accounts for 17% of the total food expenditure. India has shown impressive steady growth in the milk production which is about 15% of the total milk production in the world. India is among the world's largest and fastest growing market for milk and milk products. The average annual growth rate of milk production has been 4% during the past decades.

The lack of cooling chain facilities to keep the liquid milk fresh led to the diversion of milk for preparation of indigenous milk products with enhanced shelf life. Around 53% of milk produced in India is converted into a variety of traditional value added milk products such as heat desiccated products, cultured products, fat rich products, acid-heat coagulated products, and milk based puddings.[1] Over the years, these value added milk products are manufactured by traditional milk based sweetmeat makers who form the core of this cottage industry. This industry has played a significant role in the Indian economy. Table 9.1 gives the detailed classification and uses of traditional milk products of India.

TABLE 9.1 Classification and Uses of Traditional Milk Products of India.

Principle of manufacturing	Product name	Uses
Heat desiccation	*Khoa (Heat Desiccated)*	*Khoa* based sweets: *Burfi, Peda, Gulab-jamun, Kalakand, Milk Cake, Kunda,* and so forth.
	Rabri (Sweetened)	Direct consumption
	Basundi (Heat Concentrated)	Direct consumption
Heat and acid coagulation	*Chhana (Cheese similar to small curd cheese)*	*Chhana* based sweets: *Rasogulla (Sweet Cheese Syrup Ball), Sandesh, Rasamalai, Chhanamurki,Chan-cham*, and so forth.
	Paneer	Culinary dishes, Direct consumption
Fermentation	*Dahi*	Culinary dishes, Direct consumption
	Chakka (strained yogurt)	*Shrikhand (strained yogurt mixed with sugar), Shrikhandvadi*
	MistiDahi (Sweetened Yogurt)	Direct consumption
Fat concentration	*Makkhan (Butter)*	Direct consumption, *Ghee* making
	Ghee	Culinary purpose, Direct consumption
Frozen	*Kulfi/Kulfa (Frozen Dessert)*	Direct consumption
Addition of cereals and desiccation	*Kheer Payasam*	Direct consumption

According to Food Safety and Standards Authority of India,[7] *Khoa* is sold such as *Pindi*, *Danedar*, and *Dhap*. *Mawa* or *Kava* means the product

obtained from cow or buffalo or goat or sheep milk or milk solids or a combination thereof by rapid drying. The milk fat content shall not be <30% on dry weight basis of finished product. It may contain citric acid not more than 0.1% by weight. It shall be free from added starch, added sugar, and added coloring matter. *Khoa* is a heat desiccated, partially dehydrated milk product. It is obtained by heat desiccation of whole milk to 65–70% milk solids without the addition of any foreign ingredients, mostly in private and unorganized sectors of India. It is also known as *mawa, mava, khoya*, *khoa*, *khawa, khava, kava,* or *palghoa*. Due to its large scale consumption, more than 9 lakh tons of *khoa* is produced annually, which is equivalent to 7% of India's total milk production.

The nutrient content in terms of gross chemical composition of *khoa* is depicted in Table 9.2. *Khoa* is a rich source of energy, about 458 Kcal/100 g of the product. The food and nutritive value of *khoa* is very high. It contains large quantities of body building proteins, bone forming minerals, and furnishes with energy giving fat and lactose. It is also expected to retain most of the fat soluble vitamins A and D, and fairly large quantities of water soluble vitamins including vitamin B.[2]

TABLE 9.2 Gross Chemical Composition of *Khoa.*

Constituent	Market samples (range)	Laboratory prepared *khoa* from buffalo milk*	Laboratory prepared *khoa* from cow milk**
		%	
Ash	3–5	3.5	3.7
Fat	22–39	24.2	22.2
Lactose	17–33	22.0	24.9
Moisture	20–40	32.0	30.4
Protein	16–26	18.3	18.8

**Khoa* prepared from standardized buffalo milk (5–8% fat and 9.0% SNF) under controlled conditions.

***Khoa* prepared from cow milk standardized to 4.0% fat and 8.6% SNF.

9.2 CLASSIFICATION OF *KHOA*

Depending on the end use and the quality of milk used *khoa* is classified into three commercial types namely, *Pindi, Dhap, and Danedar.*[8] These three different varieties differ from each other in composition, texture, and quality. The requirements of the three types of *khoa*, viz. *Pindi, Danedar,* and *Dhap*

designated by ISI are given in Table 9.3 and their specific characteristics described as follows.

TABLE 9.3 Indian Standards Institution (ISI) Requirements for *Khoa* (Modified from ISI. *Specification of Khoa (IS: 4883, 1980)*; Indian Standards Institution (ISI): New Delhi, 1999.)

Characteristics	Requirements		
	Pindi	Danedar	Dhap
Chemical parameters			
Fat, min.		37.00 (% on dry basis)	
Titratable acidity, max.	0.8 (% Lactic Acid)	0.9 (% Lactic Acid)	0.6 (% Lactic Acid)
Total ash, max.		6.0 (% on dry basis)	
Total solid, min.	65(% by mass)	60(% by mass)	55(% by mass)
Microbial parameters			
Yeast & mould, max.		50 (counts/g)	
Coliform, max.		90 (counts/g)	

9.2.1 PINDI KHOA

It is formed as a disc shaped pat and has a smooth and homogenous body and texture. The grains in it are very fine and of uniform size throughout the mass. It is prepared by heating for a longer period in the pan to achieve lower moisture content. The product possesses a characteristic heated/cooked flavor. It is used for the manufacture of different varieties of *peda* (Fig. 9.1) *and burfi* (Fig. 9.2).

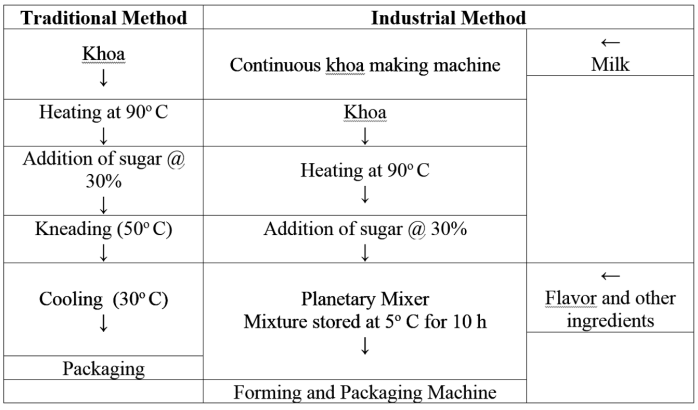

FIGURE 9.1 Flow diagram of *peda* manufacturing. (From Chavan, R. S.; Ahmadansari, M. I.; Sunil, C. K.; Chavan, S. R. *Entrepreneurship Development in Indian Traditional Dairy Products. In Entrepreneurship Development Through Agro Industrial Enterprises- Principle and Practice*; Lambert Academic Publishing GmbH & Co.: Germany, 2012; pp 555–561. Used with permission.)

Traditional Method [5]		Sugar Free Burfi [3]
Khoa ↓		Buffalo Milk ↓
Heating ↓		Filtration ↓
Adding of sugar @ 30 % of Khoa ↓		Desiccation till semi-solid consistency is reached ↓
Kneading (50° C) ↓		Cooling in tray for 15 min ↓
Whipping (50° C) ↓	←Addition of additives (Optional)	Khoa ↓
Cooling (25° C) ↓		Addition of sweetener (aspartame 0.065%) dissolved in 25 ml of milk ↓
Forming ↓		Further desiccation for 10 min ↓
Packaging		Cooling to room temperature ↓
		Packaging and storage (6-8° C)

FIGURE 9.2 Flow diagram for the manufacture of *burfi*.

9.2.2 DANEDAR KHOA

Though it is not dried but it is characterized by its granular texture and irregular shaped body. The size of grains depends upon the amount of acidulant added and the acidity of milk used. Citric acid, when added, should normally be less than 0.1% (preferably 0.02%). This type of *khoa* is used as a base for the preparation of *kalakand,* milk cake, and so forth, where granularity is desirable.

9.2.3 DHAP KHOA

This type of *khoa* is also not dried. It is characterized by a loose and sticky body, and a smooth texture. It is normally a pre-*Pindi* stage and thus contains higher moisture. *Dhap khoa* is purposely prepared for its use in preparation of *gulabjamun* (Fig. 9.3) and *pantua* so that balls of smooth surface can be prepared.

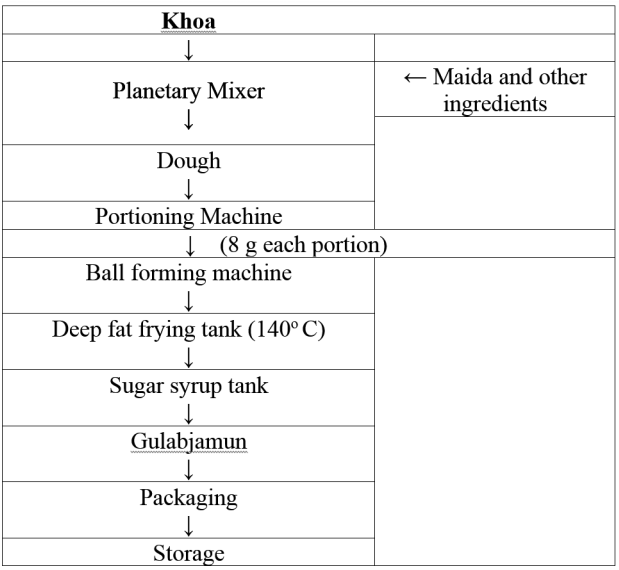

FIGURE 9.3 Flow diagram for the commercial manufacturing of gulabjamun.[5] (From Chavan, R. S.; Ahmadansari, M. I.; Sunil, C. K.; Chavan, S. R. *Entrepreneurship Development in Indian Traditional Dairy Products. In Entrepreneurship Development Through Agro Industrial Enterprises- Principle and Practice*; Lambert Academic Publishing GmbH & Co.: Germany, 2012; pp 555–561. Used with permission.)

9.3 ECONOMIC IMPORTANCE OF *KHOA*

Indian traditional milk products have played a significant role in the economic, social, religious, and nutritional well being of our people since time immemorial. It is estimated that about 50–55% of milk produced in India is converted by the traditional sector into variety of Indian traditional milk products. *Khoa* has considerable economic and dietary importance to Indian population. It forms an important base for preparation of milk sweets, which are an integral part of Indian food heritage. *Khoa* and *khoa*-based sweets have high commercial significance because of their popularity across the Indian subcontinent and longer shelf life. In India, milk sweets have been an indispensable part of the socio-cultural life. They reflect the wealth and status of people, while forming an important part of their cuisine. These are offered on festivals, special religious occasions, and social events. The market for *khoa*-based sweets is expanding overseas also. The total Indian sweet market is around 600 billion in terms of annual sales.

9.4 METHODS OF *KHOA* MANUFACTURE

9.4.1 TRADITIONAL METHOD

Conversion of milk into *khoa* is the easiest way of preserving rurally produced milk. In this method, an open iron pan evaporation process wherein locally available wood, cattle dung cake, coal, or kerosene are used in open *chulah (open fire)* as fuel for *khoa* making. Milk is heated in an open pan over a non smoky fire and continuously stirred and scraped to avoid the scorching of milk solids sticking to the pan. This method is used by unorganized traders for small scale production. It involves boiling 3–4 kg of milk leading to constant evaporation of water and the milk thickens progressively. At a certain stage of concentration (2.5 and 4 times for buffalo milk and cow milk, respectively), heat coagulation of milk proteins occurs resulting in a viscous mass, marked by an abrupt change in color. After this stage, the heat desiccation is continued with closer attention and increased speed of scraping until a semisolid consistency is obtained. The final product is ready when it shows signs of leaving the bottom and sides of the pan and starts sticking together. The pan is removed from the fire and product is worked up and down and finally it is made into a *khoa* pat before removing from the pan. Scraping is the single most important factor governing the quality of the finished product.

The *Pindi* type *khoa* is prepared by heating for a longer period in the pan to achieve lower moisture content than that of the *Dhap* type. If citric acid is added as soon as the milk starts to boil, *Danedar* type *khoa* is obtained. The *khoa* produced in such small batches usually varies in quality owing to the varying temperature profile over the heat transfer surface of the pan and the uncontrolled heat input. Intensity and speed of agitation and scraping are also varied as evaporation progresses. If the scraping is not vigorous enough, the product is likely to caramelize or burn, leading to poor market acceptability and a low price.

This traditional method of *khoa* making has following drawbacks in terms of energy consumption, cost of preparation, loss of nutrients, and quality of the product:

- Limited capacity due to batch operation, non uniform product quality and thus unsuitable for large quantity production,
- Inefficient use of energy and low heat transfer coefficient results in bulky equipment,
- It is a labor intensive method due to lengthy process time, and

- Sometimes burning of the product occur leading to poor organoleptic quality.

9.4.2 MECHANIZED METHODS

In order to overcome disadvantages of traditional methods of *khoa* making, various researchers have attempted mechanization of the *khoa* making process. Following considerations depict the technological advancement in mechanization and automation of *khoa* production process, which can be adapted for industrial scale production of *khoa*:

a. To increase the intensity of heating, a diesel-fired burner is used in place of the open wood fire. To scale up production, more than one burner is employed,
b. A double-jacketed, steam-heated stainless-steel kettle, with or without an in-built stirrer-cum-scraper, is used,
c. To overcome the problem of metallic contamination the equipments are fabricated from stainless steel,
d. A hemispherical pan joined to a cylindrical water jacket by a safety valve to control steam pressure and a rotary stirring-cum-scraping mechanism is used,[10,11]
e. Mechanized *khoa* making uses a Scraped-Surface Heat Exchanger (SSHE) in batch processing as well as continuous processing for commercial applications,[6]
f. A roller dryer, with scraper blade(s), capable of manipulating process parameters such as concentration of milk, speed of rollers, steam pressure, and flow rate is used,
g. A batch type prototype mechanized conical process vat with a sanitary type scraping mechanism having spring loaded blades for *khoa* production was developed,[6]
h. A continuous *khoa* manufacturing machine by providing three jacketed cylinders placed in a cascade arrangement,
i. *Khoa* powder was manufactured by employing reverse osmosis (RO) technique in which the steam jacketed kettle was replaced by SSHE. The product obtained by this machine gives burnt flavor.
j. A *khoa* making process was developed in which the pre-concentrated milk obtained by RO was heated in an open stainless steel steam jacketed kettle.

9.4.3 KHOA POWDER

Manufacture of *khoa* powder has been attempted to reduce the bulk of indigenous product by moisture evaporation and to extend the shelf life of the product. *Khoa* powder can be produced in the flush season for marketing in the lean season. Various processing techniques have been tried to manufacture *khoa* powder.

In one of the processes, skim milk is concentrated to 28% TS by RO. The concentrate is then standardized to 6% fat using 20% fat cream and heated in an SSHE at 127–132 °C to impart the typical *khoa* color and flavor. After cooling the heat-treated concentrate to 65.5 °C, it is finally dried over a steam-heated roller dryer to obtain *khoa* powder.

To produce tray-dried *khoa* powder, buffalo milk is heated to a suitable temperature to reduce the moisture content to about 20%. The partially desiccated *khoa* is pulverized to fine particles and uniformly spread in trays to a thickness of 1 cm. It is then dried in a drying chamber at 70 °C for 4 h or in a vacuum oven at 63.5 cm of pressure for 3 h. The concentrated *khoa* particles can also be dried in a fluidized bed dryer at an inlet air temperature of 92 °C and outlet air temperature of 80 °C.

To utilize roller dryers for making *khoa* powder, standardized BM is first heated to a predetermined temperature in a stainless steel double-jacketed vessel to develop a cooked flavor. The heated milk is pumped to the milk distribution channel of the roller dryer at a predetermined flow rate and steam pressure of 4.5 ± 0.3 bar (448 ± 34 kPa). *Khoa* powder formed in thin sheets is then scraped, ground, sieved, and packaged. Spray drying is used in the large scale manufacture of *khoa* powder. Buffalo milk is vacuum evaporated to 35% TS, heated to develop a cooked flavor, and spray dried at an inlet air temperature of 190 °C and outlet air temperature of 88 °C. *Khoa* powder can be preserved in tin containers for up to seven and nine months at 30 and 5 °C, respectively.

A comparison of all the available methods for large scale production of *khoa* indicates that the inclined SSHE gives the best results. The sensory characteristics of the product prepared by this method are reported to be similar to that of product obtained by traditional method. As there is no exposure of the product to the environment during manufacture, *khoa* made by using inclined SSHE is expected to have better microbial quality and shelf life, which can be further enhanced through packaging in a suitable packaging material under hygienic conditions.

9.5 PRETREATMENT OF MILK

9.5.1 QUANTITY OF MILK PER BATCH

A batch of 3–4 kg of milk is used in the traditional method. This amount is 20–25% of the total capacity of the pan. A lesser quantity of milk may result in burning of some milk constituents, whereas a larger quantity would be difficult to handle, particularly while boiling and may cause milk losses.

9.5.2 HOMOGENIZATION OF MILK

It produces a softer product, improves the color of *khoa* giving it a whiter appearance and reduces the amount of visible free fat present. Hydrolysis of the lactose in milk prior to its conversion into *khoa* gives a product with higher peroxide value, softer body, sweeter taste, more uniform texture, and brown color and resistance to mold growth. *Khoa* made from lactose unhydrolyzed milk exhibits higher proteolysis and lipolysis and is also susceptible to mold growth within 3–4 days of storage. Concentration of milk by RO leads to a slightly higher moisture content, significantly higher fat content and lower ash content in the product on a dry matter basis in comparison with conventionally made *khoa*.

9.5.3 HEAT TREATMENT

Heat treatment of milk in general is aimed at meeting public health requirements, water removal, facilitating mixing and blending, and imparting desirable properties to the product. Removal of water to a desirable level provides a proper body and texture and enhances the keeping quality of *khoa* by minimizing bacterial load to a negligible level and slowing down some of the chemical and biochemical reactions. The milk is kept under boiling conditions continuously until a semisolid/pasty consistency is achieved. Subsequently, the temperature is reduced to 80–85 °C until the final stage. Higher temperatures during the later stages results in an undesirable coarse texture, a brownish color, and an intense cooked/burnt flavor.

9.5.4 RATE OF STIRRING-CUM-SCRAPPING

The milk is stirred and scraped continuously to attain proper and uniform mixing, it facilitates rapid evaporation of water, avoids localized action of heat and it imparts desired body and texture into the finished product. Low speed of stirring (30–40 rpm) imparts in an undesirable appearance, texture, and flavor. Medium (100 rpm) to high (150 rpm) speed of stirring-cum-scrapping in a circular motion during desiccation is required for *khoa* making, which otherwise may lead to undesirable changes in its properties.

9.5.5 METHOD OF MANUFACTURING

Khoa made by a continuous method has a coarser texture and softer body when compared to the traditionally made *khoa*. *Khoa* manufactured from whole milk pre-concentrated (31% TS) by RO has flavor and texture comparable to that of *khoa* made in a traditional open pan. The mineral content of RO *khoa* is slightly lower owing to permeation of these compounds through the RO membrane. RO *khoa* lacks graininess and shows a slightly higher free fat and moisture content than the traditional product. The *khoa* obtained from reconstitution of *khoa* powder tends to have a pale yellow color with a brown tinge, moist surface, uniform texture, soft and smooth body, cooked flavor, and slightly salty taste.

9.5.6 YIELD OF KHOA

It depends on several factors, including the type and quality of milk, the extent of dehydration, moisture content, and handling losses (e.g., overflow and sticking residues). Moisture content is the most important factor affecting the yield of *khoa*. The average yield from buffalo milk is 21–23% and from cow milk is 17–19%.

9.6 PACKAGING, STORAGE, AND SHELF LIFE OF *KHOA*

Although packaging is one of the major factors affecting keeping quality of *khoa*, yet it has been observed for several years that it is the aspect most neglected by traditional manufacturers. This may be because of the fact that the product is usually sold at a nearby market soon after manufacture and

hence does not need a protective container. However, even when the product needs relatively longer transportation, the traditional practice of wrapping the individual *khoa* pats in leaves and then packing in a bamboo basket is still followed. Now that the product has attracted the attention of the organized sector, it is packaged in vegetable parchment paper, mechanical parchment paper (glassine), or polyethylene pouches or bags. Many manufacturers have also started using aluminum-coated laminates or tinplate cans. As *khoa* is transported to the market soon after manufacture, its storage at the production place is not given much importance when produced in smaller quantities. The hot climatic conditions prevailing in the Indian subcontinent are among the major factors responsible for the limited keeping quality of *khoa*. Therefore, refrigeration is preferred for bulk storage. *Khoa* contains about 20–30% moisture and thus falls under the category of intermediate-moisture food. This makes it highly perishable and it remains acceptable for a maximum of five days at 30 °C (ambient temperature) or for 15 days at 7 °C.

Other factors that influence the shelf life of *khoa* are (a) the microbiological quality of raw milk, (b) the water content in milk, (c) the sanitary conditions at the manufacture and storage places, and (d) the type and method of packaging. Use of vacuum packaging and gamma radiation (60 °C) to sterilize the polypacks before product packaging and addition of potassium sorbate (0.3%) at the final stage of *khoa* making have been found to be beneficial in extending the shelf life of the product. But addition of any preservatives to *khoa* is prohibited under the PFA rules.

9.7 CHANGES OCCURRING DURING *KHOA* MAKING

The most noticeable change observed during *khoa* making is the change of state of liquid milk to solid *khoa*. Heating of milk leads to denaturation and coagulation of milk proteins. It can be evidenced from change in color and consistency of the product toward the end of the manufacturing process. The whey proteins are coagulated by the action of heat, whereas the destabilization of caseins may be attributed to the combined effect of progressively higher total solids content, altered salt balance, decreased pH, and high temperature.[12] The fat globules in milk undergo appreciable subdivision owing to the vigorous agitation at a high temperature. A considerable amount of free fat is present in the final product owing to rupturing of the fat globule membrane. From a dilute solution in milk, lactose is present as a supersaturated solution in *khoa*. However, crystallization of lactose does not occur in *khoa*. A portion of the milk salts are precipitated by the action

of heat. There is a significant reduction in the total and soluble calcium, magnesium, and phosphorus in *khoa*.

During the traditional method of *khoa* making, its iron content increases to >100 mg/kg from 2–4 mg/kg in the milk. This is due to the incorporation of iron from the surface of the pan caused by the vigorous scraping during manufacturing. Caramelization due to the interaction of proteins and carbohydrates imparts a typical flavor and other sensory characteristics to *khoa*.

9.7.1 CHANGE OF STATE

The liquid milk is converted to solid *khoa* due to considerable dehydration. The water is removed and an increase in concentration of milk solids by 5–6 times takes place. The water is also dispersed as fine droplets in the mass of *khoa* and, therefore, *khoa* do not appear to be wet in the final form.

9.7.2 CHANGE IN INTENSITY OF COLOR

Milk is white in color. The color of milk changes from "light" to a more intense shade of color (with a tinge of brown). This abrupt change of color and also consistency is due to denaturation and coagulation of protein either during the process or toward the end of the process.

9.7.3 CHANGE IN MILK FAT

Due to the vigorous agitation of milk at high temperature, the fat globules are appreciably sub-divided. Considerable free fat is also produced due to the rupturing of the fat globule membrane by the vigorous scraping action of the stirrer. The vigorous agitation of hot milk has an appreciable homogenizing action so that when the stage of coagulation is reached all the fat globules are entrained in the coagulum. Almost half of the globular fat is released as free fat, the extent of which depends upon the type and fat content of milk and the manufacturing process. Usually, 44.8–62.8% of fat appears as free fat in *khoa*.

9.7.4 HEAT COAGULATION OF MILK PROTEINS

The main reaction in the preparation of *khoa* is the heat denaturation and coagulation of milk proteins. Most of the albumin and globulin are rapidly

denatured, the protective properties of the other colloids are destroyed early in the boiling process, and the process is accelerated by the incorporation of air and the frothing during stirring. Total heat coagulation of the proteins occurs when the boiling mixture thickens to a buttery consistency in the pan. Coagulation of milk protein is brought about by heating to 132—136 °C. Albumin and globulin are coagulated below 100 °C, while casein is coagulated above 100 °C. During desiccation, whey proteins are almost fully denatured while casein is also irreversibly denatured from colloidal state of non-dispersible state. The factors that influence the heat coagulation are temperature, time of holding, concentration of casein, acidity of milk, salt balance, and precise heat treatment.[13]

9.7.5 CHANGE IN LACTOSE

The lactose is present in milk in dilute solution. The lactose in *khoa* is present mostly as a super-saturated solution, since the available water is only sufficient to make roughly a 50% solution, while the solubility of L-lactose hydrate at atmospheric temperature is only 16%. In the hot *khoa* the lactose would be anhydrous but it is doubtful if any of this would crystallize out, since the drops of water are too small in size.

9.7.6 PRECIPITATION OF MILK SALT

Portions of the milk salts are precipitated by the action of heat.

9.7.7 INCREASE IN IRON CONTENT

Iron content which is about 2–4 ppm in milk, exceeds 100 ppm in the *khoa* manufactured in iron based kadhai (due to the incorporation of additional quantities of iron from the pan-surface into the finished product by the vigorous scraping of the stirrer).

9.7.8 CHANGES IN VITAMINS

Different observations are available on the changes in vitamins during processing of *khoa.* A loss of vitamin A was reported in one report in *khoa*

preparation, which could be made up by fortification of vitamin A in milk itself or by adding synthetic vitamin A to *khoa* itself. In another report a loss of ascorbic acid, thiamine, riboflavin, and nicotinic acid as well as vitamin A was reported in the course of heating of milk for making *khoa.*

9.8 FACTORS AFFECTING QUALITY OF *KHOA*

Buffalo milk is preferred for *khoa* making because it yields higher quantity of the product. *Khoa* prepared from BM is with light greenish color, soft loose body, smooth granular texture, rich nutty flavor, and slightly sweetish taste. Such *khoa* is better suited for preparation of most sweets than cow milk *khoa*, which is harder and has pale yellow color, sticky body, sandy texture, moist surface, rich nutty flavor, and slightly salty in taste. Levels of fat and the ratio of nonfat solids to fat solids in milk influence both the yield and the moisture content of *khoa*. The buffalo milk with a minimum of 5% fat or cow milk with a minimum of 4% fat is necessary to obtain the desirable body and textural characteristics in *khoa*. Milk with a lower fat content results into *khoa* with hard body, rubbery, and coarse texture. Fresh milk is preferred for better quality *khoa*. Aged raw or heat treated milk imparts a coarse texture, sour smell, and sourish bitter taste to *khoa*. *Khoa* made from goat milk is characterized by a yellow color, slightly hard body, smooth texture, and pronounced salty taste. Unlike that from cow milk or buffalo milk, the product from goat milk does not leave the sides of the heating surface easily during the final stages of manufacturing. The sticky nature of the resultant *khoa* is attributed to almost no release of free fat during its manufacture. Adulteration of milk with water lowers the yield without affecting the quality of *khoa*. The presence of added neutralizers and stabilizers gives an undesirable salty taste to the product. Adulteration of milk with starch yields puffed-up and sticky-bodied *khoa* with a starchy smell and taste.[14]

9.9 PHYSICOCHEMICAL CHANGES DURING STORAGE OF *KHOA*

After manufacture of *khoa*, microbial contamination and the interaction of milk components during the storage of *khoa* lead to many physicochemical changes. The changes occurring during storage are summarized as follows.

9.9.1 MOISTURE CONTENT

It is reported that *khoa* stored at room temperature without any preservative showed an increase in its moisture content for up to four weeks and thereafter the moisture content declines progressively throughout the storage period of nine weeks. Studies have shown that the loss of moisture during the storage varies with the type of packaging used and the temperature of storage.[15] Among the several packaging materials tested, *khoa* packed in parchment paper and in a 5-ply laminate package showed the maximum and minimum loss of moisture during storage, respectively.

9.9.2 LACTOSE AND ACIDITY

Significant breakdown of lactose occurs during the storage of *khoa*, which varies with storage temperature and the microbiological quality of *khoa*. It has been reported that the breakdown of lactose during storage at 22 °C for 9–11 days and at 37 °C for 5–7 days was about 19–60%, respectively. A progressive increase in the titratable acidity of *khoa* is observed during storage. Increase in acidity is positively correlated to flavor deterioration of *khoa*.

9.9.3 LIPOLYTIC CHANGES

The lipids in *khoa* undergo hydrolysis due to the action of microbial lipases, which causes an increase in the Free Fatty Acid (FFA) content. This influences the flavor of *khoa*. Lipolytic changes in *khoa* have been reported by several workers and usually are expressed either as the percentage of oleic acid or as the acid degree value. The liberation of FFAs was higher at 22 °C than at 37 °C. The higher concentration of FFAs at the lower temperature of storage is attributed to optimum growth of yeasts and molds. Besides the release of FFAs, the lipids in *khoa* undergo oxidative deterioration during storage and lead to the development of oxidative rancidity. The extent of oxidation of lipids in *khoa* as a function of time is measured by estimating the peroxide value.

9.9.4 PROTEOLYSIS

Proteins in *khoa* undergo microbiological degradation during storage, which causes changes in flavor, body, and texture of *khoa*. The rate of proteolysis

has been measured by various workers either by assessing the tyrosine level or by formal titration. It is observed that the rate of proteolysis in *khoa* is correlated with the bacteriological quality of *khoa* and the temperature of storage. The higher rates of proteolysis were observed at 22 °C than at 37 °C presumably because the former is the optimum temperature for the growth of yeast and molds.

9.9.5 HYDROXYMETHYL FURFURAL CONTENT

It is observed that the initial concentration of 5-hydroxymethyl furfural (HMF) in *khoa* gradually increases on storage at 30 °C. In *khoa* made from lactose-hydrolyzed milk this increase is very high at the end of four days of storage period and thereafter it declines.

9.9.6 MICROBIAL GROWTH

As milk is subjected to a very high heat treatment during *khoa* making, a low bacterial count, with very few survivors and spore formers are expected in the final product. Yet a high and varied microbiological count has been reported in market samples of *khoa* due to contamination during manufacture, handling, packaging, and storage. The general microflora of *khoa* (fresh or stored market *khoa*) has been reported to include acid producers, aerobic spore formers, proteolytic, lipolytic, and pigmented organisms, coliforms and yeasts, molds, and many other group of organisms, which on proliferation cause spoilage of *khoa*. The limited shelf life of *khoa* has been attributed to microbial spoilage. Its high nutritional value and high water activity (0.96) is conducive for the growth of bacteria.[15] Several studies carried out in different parts of India reported the presence of pathogenic organism such as *Staphylococcus aureus* and *Bacillus cereus*.[4]

9.10 DEFECTS IN *KHOA* AND THEIR PREVENTION

9.10.1 FLAVOR DEFECTS

The major flavor defects in *khoa* are sour/acid, rancid, and stale flavors. These are caused because of poor quality milk or storing *khoa* for excessively long periods. These defects can be prevented by using fresh milk, storing *khoa* at a low temperature, and early usage/marketing of *khoa*.

9.10.2 BODY AND TEXTURE DEFECTS

Low fat content of milk, low moisture content of *khoa,* and adulteration of milk with starch cause a hard body. A coarse texture is caused by high acidity and low fat content of milk, excessively high temperature and low speed of stirring-cum-scrapping. The presence of large lactose crystals due to incorrect method of manufacture causes grittiness in *khoa*. These texture defects in *khoa* can be prevented by using good-quality milk and proper manufacturing practices.

9.10.3 COLOR AND APPEARANCE DEFECTS

Poor quality milk, unclean utensils, unhygienic surroundings during manufacture, transportation and storage, and poor packaging of *khoa* lead to the presence of visible foreign particles in the product. Excessively high temperature, especially during the final stages of manufacture and inadequate stirring-cum-scraping during *khoa* making cause browning and/or presence of burnt particles in the final product. High moisture content in the product, high humidity at the storage room, and poor packaging lead to mold growth on the surface of *khoa*. A low fat content of milk cause dryness on the surface of *khoa*, whereas a high fat content and excessive stirring during manufacture causes free fat. These defects in appearance can be prevented by using good quality milk, proper manufacturing techniques and proper packaging, and storage of the product.

9.11 IMPROVEMENT OF QUALITY OF *KHOA*

Several attempts were made investigators to increase the shelf life of *khoa.* Steaming of *khoa* for 15–20 min. was reported to prevent its deterioration and shelf life is increased significantly. The keeping quality of *khoa* samples was extended by ultraviolet radiation and also by addition of cane sugar. Various approaches like use of different packaging materials, addition of preservatives and antioxidants were reported to increase the shelf life of *khoa*. The presence of moulds in *khoa* causes its fast deterioration by producing discoloration defects as well as disagreeable flavors. Efficacy of solar radiation on germicidal influence on *khoa* during storage was studied and was reported that solar radiation has a definite role in significant reduction of yeast and

moulds. Solar energy and microwaves techniques exhibited germicidal and microbiostatic properties during storage.

KEYWORDS

- ***burfi***
- ***danedar khoa***
- **dehydration**
- ***dhap khoa***
- **drying**
- ***kalakand***
- ***khoa* (condensed milk)**
- ***khoa* based sweets**
- ***khoa:* making process**
- ***khoa:* preservation**
- ***khoa:* shelf life**
- ***peda***
- ***pindi khoa***
- **reverse osmosis**
- **scraped surface heat exchanger**
- **value addition**

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APPENDIX I MILK PRODUCTS IN INDIA

Khoa

Peda

Burfi

Gulabjamun

Kunda

Kalakand

Chhana

Rasgulla

Chum chum

Rasmalai

Rabri

Basundi

PART III
Human Health Benefits from Various Dairy Products

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CHAPTER 10

MILK-DERIVED BIOACTIVE PEPTIDES: HEALTH BENEFITS

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CONTENTS

ABSTRACT

Peptides derived from milk proteins have been shown to exert beneficial effects on human health and therefore attracted the interest of researchers as a health promoting functional food. These biological properties may play an important role in the development of medical foods that treat or mitigate the effects of diseases. Bioactive peptide preparations have the potential to be used in the formulation of functional foods and cosmetics and as potent drugs having well-defined pharmacological effects. With the rise of consumer concerns about the natural components, milk-derived bioactive substances may have value in food preservation and nutraceuticals. Application of enrichment protocols such as membrane processing and chromatographic isolation may also be an area of future interest in the extraction of potent biofunctional peptides from milk and dairy products and their subsequent utilization as functional food ingredients. There is an insistent need to focus on developing novel facilities including advanced proteomics approaches, recombinant enzyme technologies, and microbial fermentation, to study the various impacts of bioactive peptides on expression of genes and also to optimize the nutritional and health effects of these compounds. Consequently allergenicity, toxicity, and stability of its biological functions during gastrointestinal digestion should be tested in formulation of products incorporated with bioactive peptides.

10.1 INTRODUCTION

It is well known that diet is one of the crucial environmental factors that influence our health and the development of certain diseases. Among the main food stuffs, milk has been identified to be of particular interest with regard to ailment and health. It is the exclusive source of nutrients for the young mammalian. Proteins are basic food ingredients. Recently, the role of protein in the diet as a physiological active ingredient has increasingly been recognized worldwide. Milk proteins are an important source of amino acids, indispensible for growth and maintenance, and a source of energy. Milk contains around 3.5% proteins, of which 80% are casein and 20% whey protein. The casein comprises αs_1, αs_2, β, and κ-casein, while whey comprises β-lactoglobulin, α-lactalbumin,[6] lactoferrin, immunoglobulins, serum albumin, glycomacropeptide (GMP), enzymes, and growth factors. Due to their role in nutrition and biological activities, milk protein

has attracted plenty of scientific interest over the years. In addition, a wide range of *in vitro* bioactivities of intact milk proteins such as satiating, antimicrobial, mineral binding, anti-lipidaemic, and anticancer properties have been reported.[2, 14, 41] Today, it has been recognized that milk protein shows numerous functionalities *in vivo* by the action of bioactive peptides. Presently, milk proteins are considered as the most vital resource of a range of bioactive peptides.[29]

Bioactive peptides are specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health.[25] Fermented dairy products and other foods containing bioactive peptides appear to have the potential to offer specific health benefits to consumers.[27] Milk protein-derived active biological constituents and bioactive peptides have received much attention for their biological significances, and are currently the subject of intensive research. The intrinsic bioactivities of the peptides encrypted in major milk proteins are latent until released and activated by enzymatic hydrolysis *in vitro* or *in vivo*.[28] Biologically active peptide fragments are formed during the degradation of the milk proteins (whey proteins and casein) by digestive enzymes in the gastrointestinal tract and by proteolytic lactic acid bacteria (LAB) during fermentation of milk. In these processes, varieties of peptides containing 2–20 amino acid residues are formed.

Depending on the sequence, these bioactive peptides may reach the small intestine intact and be absorbed as is or they may be degraded by gastrointestinal enzymes or serum peptidases in the circulation. Once liberated and absorbed, these bioactive peptides may interact with selected receptors and exert a physiological effect on the cardiovascular, digestive, endocrine, immune, and nervous systems of the body.[45] The activity of these peptides is based on their inherent amino acid composition and length of the sequence. Milk protein-derived peptides exert multiple physiological activities, including antimicrobial, antioxidant, anti-thrombotic, opioid, antihypertension activity, modulation of digestive enzymes, nutrient absorption, and immune responses (Fig. 10.1).[26, 36, 48] Many milk-derived peptides reveal multifunctional properties, that is, specific peptide sequences may exert two or more different biological activities. These regions known as "strategic zones" are partially protected from further proteolytic breakdown. Because of their physiological and physicochemical versatility, milk peptides are reckoned as very important constituents for incorporation in functional and novel foods, dietary supplements, and even pharmaceuticals with the purpose of targeting specific disease.

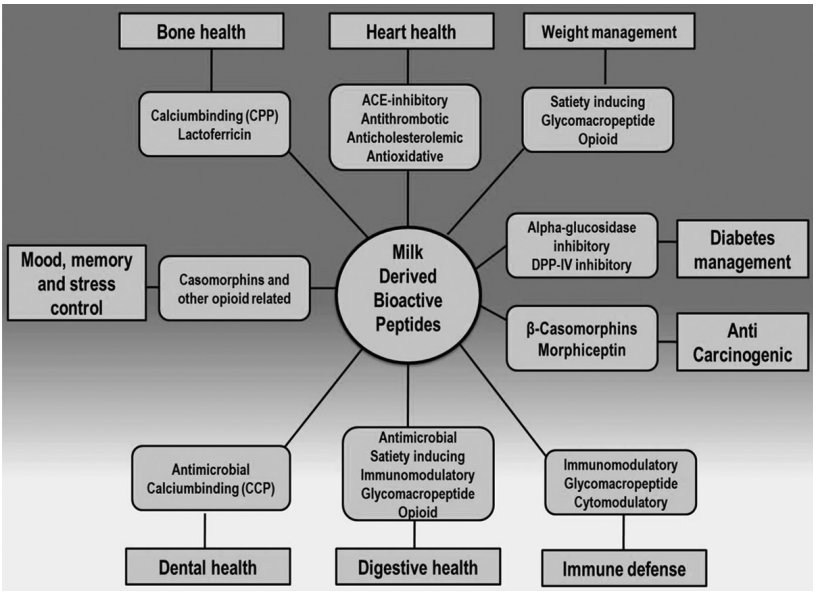

FIGURE 10.1 Physiological functionality of milk protein-derived peptides. Modified from Korhonen, 2006.[28]

In this chapter, authors have reviewed and discussed role of milk-derived bioactive peptides for benefits of human health.

10.2 PRODUCTION OF MILK-DERIVED BIOACTIVE PEPTIDES

Although consumption of low-fat milk and dairy products has a beneficial effect on the prevention or treatment of metabolic syndromes, research has been focused on milk protein-derived peptides. Milk-derived bioactive peptides can be encrypted in both casein and whey protein and can be released from their parent proteins by following four ways:[46]

a. Enzymatic hydrolysis during gastrointestinal digestion.
b. Fermentation of milk with proteolytic starter cultures.
c. Hydrolysis by enzymes obtained from microorganisms or plants.
d. Combination of fermentation and hydrolysis.

If the sequence of the peptide is known, it is also possible to synthesize peptides by chemical route, recombinant DNA technology, or enzymatic amide synthesis.[35] After releasing from origin, bioactive peptides must reach

the target receptor in the intestinal lumen or in other peripheral organs, passing via the systematic circulation. The most commonly used enzymes are pepsin, trypsin, and chymotrypsin, of animal as well as microbial origin. In cheese and fermented milk, the production of bioactive peptides results from the synthesis of metabolites with proteolytic capability by bacteria from milk-protein substrate.[19] Depending on the number and/or sequence of the amino acids, food-derived bioactive peptides can display various activities by binding to a specific receptor in the gastrointestinal tract or in target organs and tissues after absorption into the bloodstream. Figure 10.2 depicts an alternative mode of bioactive peptides generation.

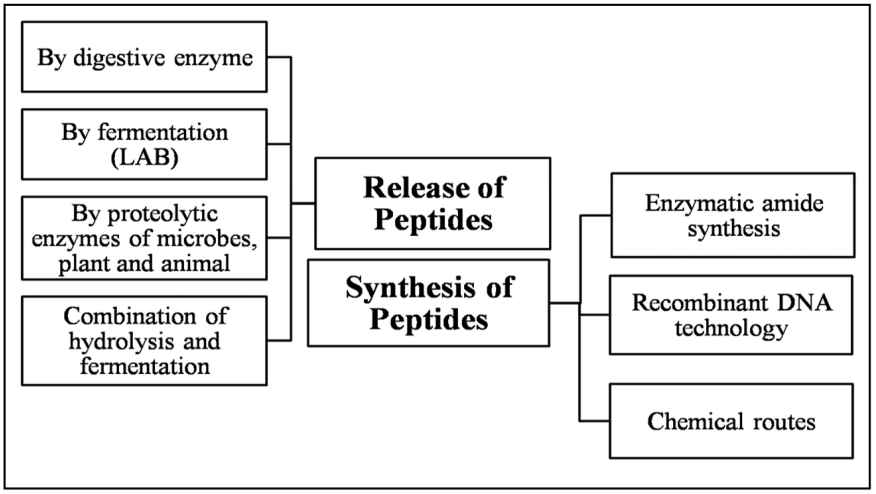

FIGURE 10.2 Modes of bioactive peptides generation: release and synthesis of Peptides. Modified from Madureira et al.[35]

In recent time, there has been a growing interest in the use of dairy hydrolysates containing bioactive peptides as agents for maintaining general health and preventing chronic human diseases. Therefore, several technologies, principally based on the enzymatic hydrolysis, have been developed for the production of these bioactive hydrolysates.[46] In the food industry, the enzymatic membrane reactor allows protein hydrolysis and the subsequent separation of peptides generated by microfiltration or chromatography techniques, such as size exclusion or ion-exchange chromatography.[58] Ultrafiltration and nanofiltration are examples of technologies that have been used to refine and isolate bioactive peptides, allowing them to be separated by size for use in specific applications. Moreover, instead of traditional methods,

subcritical water hydrolysis strategies have been proposed for dairy hydrolysates production without use of acids, bases, or enzymes. In addition, high hydrostatic pressure causes substantial modifications to milk proteins, and ultimately influences their functional properties.

10.2.1 GASTROINTESTINAL DIGESTION

During gastrointestinal digestion, physiologically active peptides are produced from several milk proteins. Hydrolysis may occur in various stages after ingestion of the protein. Dietary proteins undergo denaturation in the presence of hydrochloric acid (HCl) secreted by the parietal cells of the stomach. This acid activates pepsinogen and converts it into its active form, pepsin. Pepsin acts on proteins to metabolize them to bioactive peptides and amino acids. In the gastrointestinal tract, ingested proteins are hydrolyzed by gastrointestinal enzymes, usually pepsin, trypsin, chymotrypsin, and other membrane peptidases.[28] Other digestive enzymes and different enzyme combinations of proteinases (e.g., alcalase, chymotrypsin, pancreatin, pepsin, and thermolysin) have also been utilized to produce bioactive peptides from various proteins.[21,56] Absorption of peptides can be through the gastrointestinal wall by different mechanisms, such as by passive diffusion through the enterocytes, para-cellularly through cytosis, or through a carrier. After absorption, bioactive peptides must reach their target sites at the luminal side of the intestinal tract or at specific peripheral organs to exert their physiological effects.[10]

It has been hypothesized that peptides released from whey proteins during their transit through gastrointestinal tract might be responsible for the post-meal glycemic response produced after whey intake.[1] It has been suggested that the antidiabetic property of whey proteins is mainly due to its content of bioactive peptides which, following their release during gastrointestinal digestion, could stimulate the secretion of gut-derived hormones and/or inhibit enzymes involved in glycemic homeostasis.[23]

10.2.2 MICROBIAL FERMENTATION

LAB utilize milk protein as their key source of essential and growth stimulating amino acids. Many industrial important dairy starter cultures are highly proteolytic in nature. Bioactive peptides can, thus, be generated by the proteolytic activities of the strains of starter and non-starter LAB

in fermented dairy products. Several LAB (e.g., *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactococcus lactis* ssp. *diacetylactis*, *Lactococcus lactis* ssp.) have been reported to release bioactive peptides by the process of fermentation. Besides, to live microorganisms, proteolytic enzymes isolated from LAB have also been successfully employed to release bioactive peptides from milk proteins.[9] The production of a variety of bioactive peptides in fermented dairy products, for example, yoghurt, sour milk, and dahi, has been well documented in many studies. Fermentation of milk involves a number of metabolic pathways responsible for generating metabolites, which significantly contribute to the chemical, biochemical, and nutritional properties of the fermented products. The proteolytic system of LAB is complex and consists of three major components: proteases bound to the cell wall that promote the initial hydrolysis of milk casein into oligopeptides, specific transporters that transfer the oligopeptides to the cytoplasm, and intracellular peptidases that finish the hydrolysis process to convert oligopeptides into free amino acids and/or low molecular weight peptides.[8]

The single most effective way to increase the concentration of bioactive peptides in fermented dairy products is to ferment or co-ferment with highly proteolytic strains of LAB. The choice of strains influences the release of effective bioactive peptides. The strain should not be too proteolytic otherwise the product will be destroyed and must have the right specificity to give high concentrations of active peptides. The concentration of angiotensin converting enzyme (ACE)-inhibitory peptides seems to rely on a balance between their formation and further breakdown into inactive peptides and amino acids that in turn depends on storage time and conditions. Peptides with various biological activities can be generated during milk fermentation with proteolytic starter cultures, for example, ACE-inhibitory, immunomodulatory, antioxidative, and antibacterial peptides. As a result, peptides with wide functionalities can be found in the end-products of fermentation of dairy foods.[20, 25] Despite detailed knowledge of the proteolytic systems of LAB, information on the production of bioactive peptides, specific proteinases, and peptidases responsible for bioactive peptide release is lacking.

10.2.3 ENZYMATIC HYDROLYSIS

The most common way to obtain bioactive peptides in vogue is by enzymatic hydrolysis of protein. Proteases derived from microorganisms, animals, or plants have also been successfully employed in the proteolytic process

to release peptides from milk proteins. In addition, enzyme combinations (i.e., alcalae, chymosin, pancreatin, trypsin, and thermolysin) can be used to release bioactive peptides.[35] The commonly used enzymes of plant and animal origin are: α-chymotrypsin, papain, neutrase, thermolysin, pepsin, alcalase, pronase, carboxypeptidase A, and trypsin.[28] Generation of dipeptidyl peptidase (DPP)-IV inhibitory milk peptides by enzymatic hydrolysis from milk protein is more studied.[30, 31] Similarly, alpha-glucosidase inhibitory milk peptides have been produced from the milk protein by enzymatic hydrolysis.[30]

10.3 HEALTH BENEFITS OF MILK-DERIVED BIOACTIVE PEPTIDES

10.3.1 EFFECT ON THE CARDIOVASCULAR SYSTEM

Cardiovascular diseases have become a worldwide health problem that goes beyond socio–economic barriers and equally affects men and women. Diet plays a key role in the development of the most significant risk factors of these diseases, such as hypertension, obesity, diabetes, low-grade systemic inflammation, and atherosclerosis. In recent years, bioactive milk peptides have gained interest because of their notable antihypertensive, antioxidant, anti-inflammatory, and hypocholesterolaemic effects. In this section, the role of milk proteins-derived peptides on cardiovascular diseases is summarized and discussed.

10.3.1.1 ANTIHYPERTENSIVE PEPTIDES

Elevated blood pressure is one of the major risk factors for cardiovascular disease. ACE (EC 3.4.15.1/peptidyl-dipeptidase A) plays an important role in the regulation of blood pressure. Thus, inhibition of this enzyme is one of the strategies for the management of hypertension. *In vitro* and *in vivo* research studies have indicated that antihypertensive peptide sequences were present in both casein and whey fractions.[13] The tryptic CN hydrolysate containing the peptide αS1-CN f (23–34) has been patented and commercialized as antihypertensive peptide under the name of C12®. Another, two peptides derived from αS1-CN from pepsin casein hydrolysate with sequences RYLGY and AYFYPEL, showed potent systolic blood pressure (SBP) reducing effects in animal model.[12]

Besides, antihypertensive peptides are also produced in fermented milks and cheeses by the use of proteolytic system of LAB. For example, the production of β-CN-derived peptides, IPP, and VPP, in sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* (Calpis®) with potent SBP decreasing effects. Three groups of milk bioactive peptides with ACE-inhibitory activity have been extensively evaluated in humans; these include the CN-derived lactotripeptides (Ile-Pro-Pro and Val-Pro-Pro commercialized as Evolus® and Ameal S®), Ser-Lys-Val-Tyr-Pro and the αs1-CN-derived C12 peptide (Phe-Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys).[17] The lactotripeptides appear to be the most studied peptides in humans to date.[18]

10.3.1.2 ANTI-INFLAMMATORY PEPTIDES

Chronic inflammation is related to many age related diseases, such as atherosclerosis, vascular diseases, arthritis, cancer, diabetes, osteoporosis, dementia, obesity, and metabolic syndrome. Different cytokines play a pivotal role as mediators in the production of biomarkers implied in the progression of inflammation and the endothelial dysfunction. The down-regulation of cytokines which are responsible for the production of biomarkers implied in the progression of inflammation by using peptides may retard or alleviate inflammation, hence exerting beneficial effects against cardiovascular diseases.[53] To date, only the commercial peptide NOP-47, derived from whey, enhanced the vascular function by modulating glucose levels and biomarkers of inflammation.[3]

10.3.1.3 ANTIOXIDANT PEPTIDES

Antioxidant peptides from milk proteins play a vital role in the maintenance of antioxidant defense systems by averting the formation of free radicals or by scavenging free radicals and active oxygen species, which induce oxidative damage to biomolecules and cause aging, cancer, heart disease, stroke, and arteriosclerosis. So far, many antioxidant peptides derived from both CN and whey proteins have been characterized.[47] The peptide Tyr-Phe-Tyr-Pro-Glu-Leu from a peptidic digestion of casein had a strong superoxide anion radical-scavenging activity, attributed mainly to the C-terminal amino acid Glu-Leu. The β-casein f(169–176) fraction was found to have the greatest inhibitory effect on linoleic acid oxidation. Peptide deriving from

the β–lactoglobulin A Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile has higher radical-scavenging activity than butylated hydroxyl anisole. Some antioxidant peptides have also been isolated from fermented dairy products, such as the sequence Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met, which has been isolated milk fermented with *Lactobacillus delbrueckiissp .bulgaricus* and shows a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity.[48]

Suetsuna and Ochi[51] isolated and identified free radical-scavenging activity from peptic digest of casein. The peptide, Tyr-Phe-Tyr-Pro-Glu-Leu, was found to possess a potent superoxide anion radical-scavenging activity. The C-terminal dipeptide Glu-Leu sequence proved to be important for the activity. Caseins have polar domains that contain phosphorylated serine residues, and their characteristic sequences, -SerP-SerP-SerP-Glu-Glu, are effective cation chelators that form complexes with calcium, iron, and zinc. Thus, phosphorylated caseins and/or their peptides in the aqueous phase could be a source of natural chelators to control lipid oxidation in food emulsions by binding and partitioning transition metals away from the emulsion droplet.

10.3.1.4 HYPOCHOLESTEROLAEMIC PEPTIDES

It is necessary to maintain appropriate ratio of blood lipids, as it is one of the most important risk factors for developing cardiovascular disease (CVD). Milk proteins, particularly whey protein hydrolyzates or peptides, have been reported to exert hypocholesterolaemic effects in different animal models. The ingestion of whey protein was correlated with a significant reduction of total cholesterol levels in rats fed with cholesterol-free and cholesterol-enriched diets. A similar effect was found for the β-lactoglobulin tryptic hydrolysates in rats fed with diet rich in cholesterol. Whey protein fragment f (71–75) with sequence IIAEK, known as lactostatin, was the main factor responsible for observed effect.[40] Another peptide β-lactotensin, obtained from chymotrypsin β-lactoglobulin hydrolysate, decreased total cholesterol, LDL, and VLDL cholesterol content in mice fed with a cholesterol-enriched diet.[59]

10.3.1.5 ANTI-THROMBOTIC PEPTIDES

Thrombosis may be defined as the pathological condition in which improper activity of the hemostatic mechanism results in clot or thrombus formation in arteries, veins, or the chambers of the heart. Milk protein-derived peptides

have been described as possessing anti-thrombotic properties and their ability to prevent thrombus formation has been investigated. The peptides released from of k-casein by the action of rennin, which inhibit blood platelet aggregation and fibrinogen binding (γ-chain) to platelet surface receptors are encrypted within the sequence of GMP.[5]

The peptide from κ-CN f (113–116) has been isolated from yoghurt with anti-thrombotic activity.[15] An anti-thrombotic peptide PPK from κ-CN f (109–111), having the same structure homology with an earlier identified anti-thrombotic peptide MAIPPK which was isolated from the water-soluble extract of Spanish fermented milk drinks.[22] Fractions corresponding to κ-CN f (152–160) and f (155–160) isolated as ACE-inhibitors may also possess anti-thrombotic activity.[20] The main anti-thrombotic peptide isolated from bovine κ-casein corresponding to f (106–116) with the amino acid sequence MAIPPKKNQDK and termed casoplatelin and fragments of this peptide, known as casoplateins, such as KNQDK f (112–116) and NDQK f (113–116), are structurally and functionally very similar to the C-terminal dodecapeptide of human fibrinogen γ-chain f (400–411) corresponding to the sequence HHLGGAKQAGDV.

10.3.2 EFFECT ON THE GASTROINTESTINAL SYSTEM

10.3.2.1 MINERAL BINDING PEPTIDES

Worldwide, mineral deficiencies are the most important nutritional problems. In this context, the mineral fortification is one of the best and most common strategies to prevent this deficiency.[60] It has been proposed that the phosphorylated regions released from CN during digestion. CPPs refer to casein-derived phosphorylated peptides, which contain single and multiple phosphoryl residues, and these phosphopeptides are released by enzymatic hydrolysis of α-, β-, and κ-caseins both *in vitro* and *in vivo*.[11] CPPs have been proposed to play a role in improving the bioavailability of dietary metal ions. It has been suggested that the mineral binding properties of CPPs are linked with a specific sequence, the "acidic motif," that is, Ser (P)-Ser (P)-Ser (P)-Glu-Glu. Due to the high content of negative charges, these peptides efficiently bind divalent cations and act as biocarriers for trace elements such as Fe, Mn, Cu, and Se. CPPs generally refer to peptides generated after enzymatic treatment with trypsin and which enhance absorption of calcium across the distal small intestine.[43] CPPs are used in the food industry as ingredients or fortifiers in some low mineral-containing foods and drinks.

10.3.2.2 ANTIDIABETIC PEPTIDES

It was suggested that the antidiabetic properties of milk protein are primarily attributable to its content of bioactive peptides which, following their release during gastrointestinal digestion, could arouse the secretion of gut-derived hormones and/or inhibit enzymes involved in glucose homeostasis. One of the remedial strategies for managing T2D is to decrease postprandial hyperglycemia by retarding the absorption of glucose through inhibition of carbohydrate-hydrolyzing enzymes, for example, alpha-glucosidase, in the digestive organs.[50] Inhibition of this enzyme in the digestive tract delays carbohydrate digestion; and increases overall carbohydrate digestion time. Hence, less glucose is absorbed because the carbohydrates are not rapidly hydrolyzed down into glucose molecules and subsequently diminishing the postprandial blood glucose and insulin level. Results from one study demonstrated that peptides with inhibitory property against alpha-glucosidase activity can be generated from the peptic digestion of whey proteins.[30]

DPP-IV (DPP-IV/CD26; EC.3.4.14.5) is a multifunctional transmembrane glyco-protein. It is a 766-amino acid serine protease that contains N-terminal dipeptidases activity which selectively cleaves dipeptides after proline or alanine residues. Gastrointestinal hormones, including GIP and GLP-1, are endogenous substrates for the enzyme DPP-IV. The two incretin hormones, GIP and GLP-1, are secreted by enteroendocrine cells of the intestines within minutes of food ingested and increase nutrient-stimulated insulin secretion in a glucose-dependent manner.[16] Thus, DPP-IV inhibitors exert their positive effect on glucose regulation by slowing down the rapid inactivation of endogenous GIP and GLP-1, thus enhancing insulin secretion. Various studies have highlighted that milk protein is the natural source of DPP-IV inhibitors.[16, 54, 55] The different peptides were identified for the DPP-IV inhibitory activity of the water-soluble fraction of Gouda-type cheese. The β-casein peptide residue 70–77 (β-CN f70–77; LPQNIPPL) showed the highest DPP-IV inhibitor activity.[55]

10.3.2.3 ANTIOBESITY PEPTIDES

In regulation of food intake, satiety plays an important role and has significance in the control of obesity. Reducing food intake and increasing energy expenditure are a means to control body weight. A reduction of food intake may be facilitated by increasing satiety. Satiety, which arises from various signals between the gut and brain, corresponds to the hunger alleviation seen

after food intake. It is very well accepted that protein is the most satiating component of food. The satiating effect of whey protein is mainly due to a high concentration of branch chain amino acids, particularly L-leucine. Regarding the casein fraction of milk, it was proposed that peptides from casein hydrolysates activate the peripheral opioid and cholecystokinin (CCK) receptors and block the antagonist receptors which reduce their effect on food intake.[22] Several studies showed that the whey protein-derived peptide that is GMP stimulates the release of CCK, which may promote satiety in rats.[42] Some peptides derived from casein and from whey proteins may stimulate the synthesis of the glucagon-like peptide-1, which suppresses the intake of food by increasing the sensation of satiety.

10.3.2.4 ANTIMICROBIAL PEPTIDES

The antimicrobial activity of milk is due to the synergistic activity of naturally occurring peptides and defense proteins besides immunoglobulins, such as lactoferrin, lactoperoxidase, and lysozyme. The first antimicrobial peptide isolated from milk by the action of rennet, termed lactenin, was identified by Simmes and Jones in 1930. This peptide exhibited antimicrobial activity against pathogenic strains of streptococci. The peptide called casecidins from chymosin treated casein hydrolysates exhibited antimicrobial activity against pathogenic *Staphylococcus aureus* and several lactobacilli. Consequently, the antimicrobial peptide termed isracidin corresponding to αs1-casein f (1–23) inhibited the growth of *S. aureus* and *L. monocytogenes*.[33] An amphiphilic and a positive charge of the peptides are the important character determining the interaction with bacterial membranes. Lactoferricin is one of the most potent antimicrobial peptides from the whey protein lactoferrin and antimicrobial properties can be related to tryptophan/arginine rich proportion of the peptide.[57] The antimicrobial activity of the peptides might be due to disruption of microbial membranes, leading to ion and metabolite leakage, depolarization, disruption of membrane coupled respiration, and ultimately cell death.[44]

10.3.3 EFFECT ON THE IMMUNE SYSTEM

10.3.3.1 IMMUNOMODULATORY PEPTIDES

The functions of the immune system include recognition of pathogens or foreign materials and mounting a response to eliminate it. Initially, exposure

to a foreign pathogen affects the innate, nonspecific immune response. In this process cytokines may be released. Cytokines (pro- and anti-inflammatory) are regulators of host responses to infection, inflammation, and trauma. Cytokines inhibit the synthesis of interleukin-1 (IL-1), tumor necrosis factor (TNF), and other major pro-inflammatory cytokines. Milk protein hydrolysates and peptides enhance immune cell functions by lymphocyte proliferation, antibody synthesis, and cytokine regulation. Several peptides were identified, namely f (63–68) and f (191–193) from bovine β-casein and f (194–199) from bovine αs1-casein which stimulate phagocytosis in mice and humans *in vitro* and protect against *Klebsiella pneumonia* infection in mice *in vivo*.[52] Kayser and Meisel[24] reported that di- and tri-peptides like Tyr-Gly and Tyr-Gly-Gly from bovine k-casein and α-lactalbumin, respectively, significantly increased the proliferation of human peripheral blood lymphocytes *in vivo*. It is found that CN, whey proteins, and their different hydrolysates show modulatory effects on the immune system in both *in vitro* and *in vivo* studies. Furthermore, immune peptides formed during milk fermentation have been shown to contribute to the antitumoural effects observed in many studies with fermented milks. *Lactobacillus helveticus* fermented milk has demonstrated immunomodulating effects on lymphocyte proliferation *in vitro*[32] and the ability to stimulate the phagocytic activity of pulmonary macrophages. This strain is known to have high proteolytic activity, causing the release of oligopeptides from digestion of milk proteins.

10.3.3.2 CYTOMODULATORY PEPTIDES

Cytomodulatory peptides inhibit the growth of cancer cell and stimulate the activity of immune competent cells. The peptidic fractions from milk fermented with *Lb. helveticus* showed cytomodulatory effects in mice.[34] Additionally, cytomodulatory effects in a human breast cancer cell line were reported from the peptides obtained from the milk fermented with probiotic microorganisms. In addition, bovine skimmed milk hydrolyzed with cell-free extract of *Saccharomyces cerevisiae* reportedly exhibited anti-proliferative activity against human HL-60 leukemia cells.[49] Cytomodulatory peptides have been isolated from a variety of fermented dairy products. Dialysate and anion exchange fraction of yoghurt showed significant inhibitory action against tumors in a mouse assay on cultured mammalian intestinal Caco-2 and IEC-6 cells. Anti-proliferative peptides have also been isolated from Gouda cheese and these peptides reported to inhibit leukemia cells.[37]

10.3.4 EFFECT ON THE IMMUNE SYSTEM

10.3.4.1 OPIOID PEPTIDES

The active ingredient in opium, named morphine after the Greek god of dreams, was isolated by Serturner in 1803. Several chemical classes show morphine-like pharmacological effects, such as the phenylpiperidines, benzomorphans, and octahydroisoquinolines, and these are termed opioid drugs and defined as "agents that bind to or otherwise influence opioid receptors."[7] Opioid receptors are integral membrane proteins of the central nervous system thought to be responsible for mediating effects such as the analgesic effect, feelings of euphoria, myosis, constipation, and changes in the endocrine immune system produced by ingestion of opioid drugs in man. At least three types of opioid receptors are known to date; these are termed μ-(morphine), δ-(enkephalin), and κ-(dynorphin) receptors. The caseins (αs1, αs2, β, κ) and whey proteins are potential sources of opioid peptides. β-casomorphins, the first identified opioid peptides initially described in the bovine β-casein sequence, correspond to fragments of the β-casein sequence 60–70 corresponding to the amino acid sequence f (Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu) and are the most extensively studied peptides to date.[11]

10.3.4.2 RELAXING PEPTIDES

Among various compounds, the peptides released during digestion can be important for sedative and calming properties of milk. In fact, α-casozepine (YLGYLEQLLR), a peptide derived from tryptic hydrolysis of aS1-CN, with benzodiazepine-like activity has shown anticonvulsant and anxi-olytic activities in rats.[39] A fragment, corresponding to sequence αS1-CN f (91–97) could be the responsible for the *in vivo* activity of α-casozepine.[4] Oral intake of an encapsulated αS1-CN tryptic hydrolysate, containing this peptide, before a stressing situation, decreases the blood pressure increase induced by the stress. Moreover, the plasma cortisol concentration decreased in the treated subjects compared with those who have taken a placebo.[38] In another study, the oral intake of the encapsulated hydrolysate by female volunteers significantly reduced their digestive, cardiovascular, intellectual, emotional, and social stress-related symptoms.

10.4 FUTURE PROSPECTS

Beyond their well-known nutritional value, milk proteins may exhibit a plethora of biological activities that influence the growth, development and function of specific organs, metabolic responses to absorbed nutrients, and defense systems, among others. Many of these activities could be exerted by peptides released from parent protein during gastrointestinal digestion or food processing. The research area of bioactive peptides is only at its beginning and more sequences along with additional physiological effects will be discovered in the future. Most of the properties of milk bioactive peptides have been demonstrated by *in vitro* assays and/or animal model systems. However, data obtained from these studies are insufficient to demonstrate efficacy of those peptides in human. To generate evidence of these beneficial effects, further long-term human trials using sufficient number of subjects, controlled doses, and formulations are needed. Bioavailability of food peptides is also crucial in these studies, since it is dependent of numerous factors such as the food matrix, food composition but also it may vary between individuals. Finally, it has to be taken into account that the pharmacological approach is not always applicable to food bioactives where the physiological effects are smaller. Scientific progress in the field must be targeted at a better understanding of how these food-derived peptides interact with the human body and can prevent the initiation, development, or progression of risk factors for diet-related chronic diseases.

KEYWORDS

- **antidiabetic peptides**
- **antimicrobial peptides**
- **cytomodulatory peptides**
- **fermentation**
- **functional foods**
- **immunomodulatory peptide**
- **lactic acid bacteria**
- **metabolic syndromes**
- **milk**
- **mineral binding peptides**

- **obesity**
- **peptides**
- **protein**
- **relaxing peptides**
- **starter culture**

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CHAPTER 11

POTENTIAL APPLICATION OF PROBIOTICS FOR HUMAN HEALTH BENEFITS: REVIEW

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ABSTRACT

The use of spores as probiotics appears to be expanding, with a growing number of products commercially available. In most countries, products marketed as pharmaceuticals must meet pre market criteria for efficacy and safety and it is equally clear that supposedly safe species cannot be taken for granted and every product must be evaluated on a case-by-case basis. In general, current government legislation does not provide definitions regarding the suitability of various bacteria for food and supplement use in developed countries as well as in India. *Clostridium butyricum* MIYAIRI 588 has been safely used as a probiotic for many years. Preventive effect of CBM preparation on the abnormalities of intestinal microflora due to *Helicobacter pylori* eradication therapy was not known. The mechanism by which CBM control diarrhea is by many ways and we only know few mechanisms and much work can be done in this area, and much remains to be done to substantiate the claim of spore formers as probiotics. Studies are urgently needed to further improve our understanding of the mechanisms of enterococcal pathogenesis, which should contribute to a better distinction between pathogenic and non-pathogenic strains. It is suggested that the use of *enterococci*, as commercial probiotic strains, should be possible on the basis of case-by-case studies, establishing their innocuity or at least the lack of acquired antibiotic resistance genes and proven virulence factors *in vivo*. Despite the successful therapeutic applications of *Escherichia coli Nissle* 1917, only limited information is available about the beneficial traits contributing to the strain's probiotic character. The mechanisms underlying the probiotic nature especially at the molecular level yet have to be elucidated. To obtain possible links of these peculiarities to the probiotic nature of this strain the function and physiological roles of these and other, *E. coli Nissle* 1917 specific CDSs need to be experimentally studied.

Further investigations into the taxonomic classification of the new taxa (*Weissella)* should take place to confirm the taxonomic status of the organism *Weissella*. Work should also focus on the clarification of the full amino acid sequence of weissellicin 110 and its possible application as a biopreservative. *Weissella confusa* PL9001 has a dual inhibitory activity on *H. pylori*, bactericidal activity, and prevention of adherence to gastric mucous cells. This has the potential to be developed as a new probiotic for the stomach by performing case-by-case research to establish the bio safety of the organism. *Saccharomyces boulardii* activities in the prevention and/or the treatment of diarrhea have been widely investigated and demonstrated. This yeast is frequently prescribed in a lyophilized form as a biotherapeutic

agent. However, new data and further experimental studies should permit to better elucidate the mechanisms of action of the yeast and suggest new therapeutic applications.

The mechanisms by which *Butyrivibrio fibrisolvens* bacteria inhibit colon cancer is still a mystery. Oral administration of the live *B. fibrisolvens* MDT-1 cells to mice suppressed ACF formation. The mechanisms underlying this event are presently unclear, remain to be clarified. Further research has to be carried out to find out these aspects and many trials have to be done before introducing this new strain as a commercial probiotic. Gene technology will certainly play a role in developing new strains, with gene sequencing allowing for an increased understanding of mechanisms and functionality of probiotics. The establishment of standards of identity for probiotic containing food products will serve to accelerate the development and availability of these food products. According to Barry Goldin, Ph.D., Professor at Tufts University School of Medicine, "The full potential of probiotics can only be realized when their benefits can be established scientifically. It is highly likely that benefits from current and future probiotics have gone undetected and, therefore, full utilization of these organisms has not been achieved".

11.1 INTRODUCTION

Functional foods are gaining importance in present day health conscious world. Among various functional foods, foods containing "probiotics" are considered as an important segment.[79] Prolific research is being done on probiotics ever since Elie Metchnikoff gave the hypothesis that organisms are responsible for the longevity of an ethnic person. Lilly and Stillwell[52] first coined the term "probiotics" and defined as microorganisms promoting the growth of other microorganisms. Later Parker (1974) described probiotics as "*organisms and substances which contribute to the intestinal microbial balance.*" Subsequently probiotics have been defined in different ways. Holzapfel et al.[32] defined "*Probiotics as viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the GIT.*" IDF[37] defined "*Oral probiotics living micro-organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition.*" The field of probiosis has emerged as a new science with applications in farming and aquaculture as alternatives to antibiotics as well as prophylactics in humans.

Apart from the most widely documented and universally used species of probiotics (*viz.Lactobacillus* sp. and *Bifidobacterium* sp.), there are other bacterial species with probiotic potential, which are presently being used or being tested for their safe utilization. Among them, the prominent are: Bacillus sp., *Weissella* sp., *Enterococcus* sp., *Clostridium* sp., *Butyrivibrio* sp., and yeasts.

In this chapter, authors discuss and review the potential and applications of probiotics for benefits of human health.

11.2 BACILLUS SPECIES

Bacillus species are saprophytic gram-positive bacteria common habitat of soil, water, dust, and air. They have endospore forming capability under stress or reduced nutrients, which is resistant to different physic chemical attributes.[46] This ability is mainly due to dipicolinic acid, which forms a thick glass like structure along with different layers of protein, which makes it resistant.[83] Additionally very low moisture content in the spore also contributes to resistance which allows them to survive unfavorable conditions like acidic conditions and bile present in the human digestive system, food manufacturing processes, and during storage.[18]

There are nearly 288 recognized species of the genus *Bacillus*, among them bonafide species being used as probiotics are: *B. subtilis*, *B.cereus*, *B.licheniformis*, *B. pumilus*, *B. clausii,* and *B. coagulans*[33,74] *Bacillus* species are not natural inhabitants of the gut, but they enter into association with food. *Bacillus* sp. when used as probiotics is administered in spore form, which can resist the unfavorable conditions arising during transit through the gastrointestinal tract of humans and animals. Germination of spores and their survivability in the gut was indicated by some animal studies done on mice, pigs,[50] and in the dogs.[11]

APPLICATIONS

Probiotic strain of *Bacillus subtilis* is of wider interest because of its safety and its antagonistic effect *in vitro* and *in vivo* against different human bacterial pathogens such as *Campylobacter* sp., *E. coli*, *Staphylococcus aureus*[1] and *H. pylori*, which is due to the secretion of antibiotic like substances. It also plays an important role as an immunostimulatory agent in a variety of diseases.[22] *B. subtilis* has proven safe for human consumption and can be

given as food supplement in oral form[34] through frozen milk products[43] and also through fermented corticated soybean meal that exhibited inhibitory effect against lung cancer cells.[94]

Bacillus cereus is another organism which is widely used as a component in different drugs for humans. It has showed inhibition of growth, of individual test strains belonging to *Bacillus licheniformis, B. subtilis, Enterococcus faecalis, E. coli, S. aureus, Lactobacillus helveticus,* and *Lactobacillus casei* species due to production of bacteriocin type products[88] Probiotic CenBiot (Centro de Biotecnologia of Universidade Federal de Pelotas, Rio Grande do Sul, Brazil) prepared with a strain of *B. cereus*, fulfilled the requirements to be used as probiotic; and as spores showed D_{80} of 14 h. It also inhibited *E. coli* and *Yersinia pseudotuberculosis* after 24 h in associative culture, while being innocuous for suckling and adult mice and were not inhibited by antibiotics at low concentrations. Bactisubtil is manufactured by French firm and contains *B. cereus* IP5832.

Bacillus clausii strains demonstrated microaerophilic growth, but only some grew anaerobically in a nitrate medium. The spores were able to germinate and grow in the presence of conjugated bile salts (up to 1%, w/v) or free bile salts (0.2%) and also exhibited tolerance for the combined acid–bile challenge.[16] *B. clausii* bacterio-therapy reduces the incidence of the most common side effects related to anti *H. pylori* antibiotic therapy compared with placebo.[62] *B. clausii* spores also exerted immunomodulatory effect at nasal level by cytokine pattern in allergic children with recurrent respiratory infections.

Bacillus polyfermenticus is used clinically in East Asia since long times for treating intestinal disorders. It was first isolated by Dr. Terakado in 1933 from aerial samples and was later commercially made available in Japan and Korea. This organism showed resistance to all the intestinal enzymes and fluids due to its endospore-forming characteristic that contributed for its prolonged survival in the gastrointestinal tract.[48] *B. polyfermenticus strains* also produce bacteriocins, which showed antimicrobial property against gram-positive, gram-negative, yeast, and molds and also had antilisterial properties.[9] Kim et al.[42] demonstrated that oral administration of *B. polyfermenticus* to humans has stimulated immunoglobulin (IgG) production and helped to improve the number of CD4+, CD8+, or natural killer cells which helps in alleviation of gastrointestinal disorders.

Tests were carried out on various human colon cancer cells, including HT-29, DLD-1 and Caco-2 cells proved that *B. polyfermenticus* can be used in the prophylactic treatment for colon cancer due to its anti-tumor

properties by suppression of ErbB cells (especially ErbB2. ErbB3 cells), which act as receptors and play a major role in tumor development. This organism also has the capability of healing wounds in human intestines by triggering the production of proangiogenic cytokines IL-8 and neutralizing antibodies against IL-8 or IL-8 receptor CXCR2 reduced tube formation as well as actin-stress fiber formation.[36]

Bacillus indicus was found to produce caroteniods and also can be used as a food supplement.[34] Oral dosage of *Bacillus oligonitrophilus* have shown to prolong the life of cancer patients and stabilized the cancer growth.[57] *Bacillus* CIP 5832 is also the active ingredient in Bactisubtil (Merrell, Neuilly-sur-Seine, France) a drug approved in France in the 1950s and recommended in infants against antibiotic-induced diarrhea.[51]

11.2.1 CLASSIFICATION OF PROBIOTIC SPORE PRODUCTS

The products manufactured using bacillus spores fall into two major groups: those for prophylactic use and those sold as health food supplements or novel foods.

11.2.1.1 PROPHYLACTIC USE

Among the large number of probiotic products in use today are bacterial spore formers, mostly aerobic rods of the genus *Bacillus* used primarily in their spore form. One of the oldest products on the market and available in Italy since the 1950s is Enterogermina (*B. clausii*), which is poly antibiotic resistant in nature. Other well-known products like Bactisubtil, Paciflor C10 (*B. cereus* termed IP5832), Biosporin (B. *subtilis* and *B. licheniformis*), Biosporin (*B. subtilis* strain 3 or 2335) Biosporin, Subalin, Biosubtyl NhaTrang (*Bacillus pumilus*), Biosubtyl Da Lat (*B. cereus*), Subtyl (*B. cereus*) and Bibactyl (*B. subtilis*) Biscan *B. polyfermenticus* SCD an invalid species name are being produced.[33] Bio-Three another drug developed by admixture of *B. mesentericus* , *C. butyricum,* and *E. Faceless* was found to promote stimulating the Th1 immune response, down regulate pro-inflammatory cytokines (TNF-α) and up-regulate anti-inflammatory cytokine (IL-10).[35] Table 11.1 shows list of *Bacillus* containing products marketed around the world region wise.

TABLE 11.1 List of Bacillus Containing Products Marketed Around the World

Product Name	Organism(s) present
Bactisubtil	*B. cereus*
Bibactyl	*B. subtilis*
Bidisubtilis	*B. subtilis*
Bifilac	*Clostridium butyricum, Bacillus mesentricus, Lactobacillus sporogenes*
Binifit	*Streptococcus faecalis, Clostridium butyricum, Bacillus mesentricus*
Biosporin	*Bacillus subtilis; Bacillus licheniformis*
Biosubtyl	*B. pumulis*
Biosubtyl	*B. cereus*
Biosubtyl	*B. pumilus*
Biosubtyl DL	*B. subtilis and L. Acidophilus*
Biovicerin	*B. cereus*
Bispan	*B. polyfermenticus*
Domuvar	*Bacillus clausii*
Enterogermina	*Bacillus clausii*
Flora-Balance	*Brevobacilluslaterosporus*
Lactipan Plus	*Bacillus subtilis*
Medilac Human	*B. subtilisstrain RO179 and E. faecium.*
Nature's First Food	*B. subtilis, B.polymyxa, B.pumilusand B. Laterosporus*
Neolactoflorene	*L. acidophilus, B. bifidum and L.sporogenes.*
Pastylbio	*B. subtilis*
Primal Defense	*B. subtilis and B. licheniformis*
Subtyl	*B. cereus varvietnami*

11.2.1.2 HEALTH FOODS AND DIETARY SUPPLEMENTS

Natto, a fermented food made by cooking soybeans with *B. subtilis* (natto) or *B. subtilis* var. *natto*.[33]

11.2.1.3 THERAPEUTIC PRODUCTS

Bacillus probiotics are also being developed for topical and oral treatment of uremia in human beings. Kibow Biotech (Philadelphia, USA; www.kibow-biotech.com) has developed *B. coagulans* probiotics for the treatment of gastrointestinal infections based on a number of PCT (Patent Cooperation Treaty) which was later patented (European Patent no. EP 0986314A1,1998).

11.2.2 NEGATIVE EFFECTS OF BACILLUS SPECIES

Safety problems would only be expected from probiotic *Bacillus* strains which belong to the facultatively pathogenic genus. Strains from this genus have been described as opportunistic pathogens of animals and humans. *B. cereus, subtilis*, and *licheniformis* are sporadically isolated from mastitis, pneumonia, urogenital infections, enteritis, and septicemia, but mainly in animals and humans subjected to prolonged therapy, injuries, surgical treatment, poor hygiene, or spoiled food.

The use of probiotics will not raise any particular risk, because bacilli are widely distributed in the natural environment. Data on the pathogenicity of *Bacillus* strains must be given special attention in the dossiers. *Bacillus* strains must not produce enteric or emetic toxins, when assayed in reliable test systems. Bacillus infections linked to probiotic consumption include three reports detailing seven cases of *B. subtilis bacteremia*, septicemia, and cholangitis, all in patients with underlying disease.

The use of *Enterococcus* and *Bacillus* strains (even when they do not carry transferable resistance genes) may be problematic and should be accepted only for clearly defined strains which have been tested negative for toxicity and pathogenicity *in vitro* and *in vivo*. *B. indicus* carried resistance to clindamycin at a level above the minimum inhibitory concentration breakpoints set by the EFSA. Plasmids could not be recovered from *B. indicus* so it is unlikely that this resistance could be transferred by conjugation.[34]

11.2.3 MISLABELING OF BACILLUS PROBIOTIC PRODUCTS

Hoa et al.,[31] found that majority of the commercially available probiotic preparations do not contain the labeled organism. China and India are also producing different probiotic products and the origin and status of these products appears to be poorly defined, this can be proved by the mislabeling of the product with *L. sporogenes* which in turn is taxonomically proved to be *B. coagulans*.[90] Members of the *Sporolactobacillus* group were mislabeled as *B. subtilis* (Enterogermina). Enterogermina was most closely related to members of the subgroup *Bacillus alcalophilus*[56] which can tolerate alkaline environments. *B. subtilis* was found to be consisting to the strain of *B. alcalophilus*[29] Duc et al.[22] have performed characterization of three commercially available products to verify their claim to be probiotics, and found that *B. cereus* could produce enterotoxins under favorable conditions, which are harmful to health (Table 11.2).

TABLE 11.2 List of Products Which Are Mislabeled.

Commercial Name	Labelled species	Actual species
Bactisubtil	*B.subtilis*	*B.cereus*
Biosubtyl "Dalat"	*B.subtilis*	*B.cereus*
Biosubtyl "NhaTrang"	*B.subtilis*	*B.pumilus*
Domuvar	*B.subtilis*	*B.clausii*
Domuvar	*B.subtilis*	*B.clausii*
Enterogermina	*B.subtilis*	*B.clausii*
LactipanPlus	*L.sporogenes*	*B.subtilis*
Lactiplan plus	*Lactobacillus sporogenes*	*B.subtilis*
Lactospore	*Lactobacillus sporogenes*	*B. coagulans*
Subtyl	*B.subtiilis*	*B.vietnami*

11.3 WEISSELLA

Genus *Weissella* was proposed for the first time by Collins and associates in 1993. This was based on differences that the phylogeny of the bacteria classified currently under this genus (*Weissella)* was clarified from *Lactobacillus* based on 16-s RNA studies which consists of a group of heterofermentative *Leuconostoc* like lactic acid bacteria.[32] Members of the genus *Weissella* are gram-positive, non-spore-forming, hetero fermentative and non-motile, short rods with rounded to tapered ends or coccoid in shape, occurring singly, in pairs or in short chains. *Weissella* strains have been isolated from a variety of sources and some of them play important roles in

fermentation. It is proposed that the following 12 species be included in the genus: *W. confusa*, *W. halotolerans, W. hellenica, W. kandleri, W. minor, W. paramesenteroides, W. viridescens*, *W. thailandensis*, *W. cibaria, W. kimchii*, *W. soli*, and *W. koreensis*. However, no strain in this genus has yet been commercially developed as a probiotic.[12]

W. confusa PL9001/*Weissella kimchii* strain PL9001

W. confuse (previously known as *Lactobacillus confuses* and originally known as *Lactobacillus coprophilus*) organism isolated from infant feces was identified as *W. confusa* PL9001 later renamed as *W. kimchii* strain PL9001 due to similarity of 16S rRNA.[95] It has been isolated worldwide from a variety of foods such as Greek salami[73] and Malaysian chili bo.[49] It has inhibitory effect against *H. pylori*. It has good adhesion properties. It also has capability to degrade sulfur containing materials which leads to increase in ACE inhibitory properties.[97] The bacteriocin(s) produced by PL9001 seems to belong to the class II bacteriocins.[38] Yeonhee[95] also reported that *W. kimchii* strain PL9001 can be used for the reduction of dental caries and inhibit *Streptococcus mutans*. *W. kimchii* PL9023 was observed to possess probiotic properties by inhibiting growth and adherence of disease causing vaginal isolates like *Candida albicans*, *E. coli*, *S. aureus,* and *Streptococcus agalactiae* thus promoting vaginal health.[96]

WEISSELLA CIBARIA

W. cibaria was first described by Bjorkroth et al.,[12] and can be found in various kinds of fermented foods.[20] It has good ability to withstand acid, bile, and heat tolerance. Its adhesive properties to intestinal epithelial cells are at par with *Lactobacillus rhamnosus* GG (LGG), and showed inhibitory activity against pathogens like *E. coli*, *Listeria monocytogenes*, *S. aureus*, and *Salmonella enteritidis.* It showed better immuno-modulatory effects than LGG and these results support that this organism is a profitable candidate for probiosis.[4]

Weissellicin is a bacteriocin produced by this organism. Weissellicin 110 showed a narrow spectrum of inhibition of other LAB. Unlike most class II bacteriocins produced by LAB, weissellicin 110 had no activity against *Listeria monocyotogenes.* Weissellicin 110 was found stable after high-temperature treatment.[81]

11.4 ENTEROCOCCI

Enterococci as a group was first described in 1899 and was proposed as a new genera by Thiercelin and Jouhaud.[87] Among this group *E. faecium* and *E. faecalis* can be useful as a probiotics for both humans and animals, however *E. faecalis* is used mostly for animals.[7]

ENTEROCOCCUS FAECIUM

They are found as normal inhabitants of the oral cavity, gut flora (10^5–10^7 CFU/g of stool) and upper genital tract of man and also in diverse habitats like in plants, birds, reptiles, insects, soil, and water. These are spherical to ovoid cells chiefly in pairs and short chains. Elongated cells may be formed. Non-motile and motile strains are also reported. They are strictly anaerobic strains which can adopt to grow aerobically have been isolated from bovine alimentary track.

E. faecium strains used as probiotics efficiently protect animals from diseases caused by *E. coli*, *salmonellae* or *Clostridia*. Report show that the best preventive effectivity of *E. faecium* from commercial probiotic preparation Vitacanis (composed of *L. acidophilus*, *E. faecium,* and *Saccharomyces cerevisiae*) against *Salmonella enterica serovar typhimurium* in the gastrointestinal tract of gnotobiotic mice was observed. *E. faecium* was found to colonize in the human gastrointestinal tract, showing the ability to withstand the harsh conditions existing in the GI tract.[67] Enterococcus strains were found to alleviate different types of diarrhea, cholesterol lowering effect and immune regulation. Among enterococci, only *E. faecium* of human origin is used as a commercial probiotic (e.g., Probican paste; Medipharm Ltd.) (Mego et al., 2005),[59] other commercial products are *E. faecium* SF68® (NCIMB 10415, produced by Cerbios-Pharma SA, Barbengo, Switzerland) and *E. faecalis* Symbioflor 1 (SymbioPharm, Herborn, Germany).

ENTEROCOCCUS FAECIUM NCIMB 10415

E. faecium SF68 (NCIMB 10415) is a LAB, originally isolated from a healthy Swedish baby. The intestinal bacterium *E. faecium* NCIMB 10415, also known as *E. faecium* SF68, is the active ingredient of some probiotic products for humans. EU-authorized probiotic strain *E. faecium* NCIMB 10415. It was reported that *E. faecium* strain SF68 effectively treats chronic

hepatic encephalopathy and acute diarrhea as well as antibiotic associated diarrhea in humans. The strains SF68 exert inhibitory effects against important enteropathogens.[70] This strain has already been shown to invoke effects on piglet performance and on general composition of the intestinal microbiota as well as modifying the immune response of piglets.[100] Furthermore, an enterocin of this strain was shown to inhibit growth of *E. hirae*, *E. casseliflavus*, as well as 4 of 12, *E. faecium* strains, but only one of 10 *E. faecalis* strains[89] It was also seen that it is very effective in treatment of *Giardia* infection where in it stimulates the immune response system and eliminates trophozoites in the small intestine and maintains intestinal microbial balance.[8] *E. faecium* SF68 and active component when administered to children alleviated the severity and hospital stay in case of acute diarrhea.[17] It was found to reduce the antibiotic associated diarrhea, irritable bowel syndrome, and immune regulation. *E. faecium* SF68 can be decided as one of the best-documented and effective probiotics for diarrhea.

E. FAECIUM M-74

The administration of probiotic strain M-74 can be considered to be an effective and promising method for elimination of pathogenic bacteria in the case of inflammatory bowel disease and colon cancer in humans.[59] *E. faecium* M-74 was shown to enhance nonspecific immunity *in vitro and in vivo*.[23] It was demonstrated that milk fermented by *E. faecium* M-74 exhibited dose-dependent inhibition of selected mutagens and UV- irradiation in the *Salmonella* and *Euglena* assays.[7] *E. faecium* M-74 enriched by selenium, and MRS (+Se) extract after the cultivation of this strain possessed higher antimutagenic activity. It has inhibitory effects against important enteropathogens, including enterotoxigenic *E. coli*, *Salmonellae*, *Shigellae*, and clostridia. However, studies confirming the inhibitory effect of probiotics *in vivo* are still missing.[59] Probiotic strain *E. faecium* M-74 with organic selenium has an important immunostimulatory and antimutagenic properties *in vitro* and also *in vivo*.[7] A fermented milk product Gaio® (yogurt) that contains *E. faecium* (MD Foods, Denmark) has been sold in Denmark and in the United Kingdom since several years. The manufacturer claims the product has a hypocholesterolemic effect on individuals. In a study carried out by Bertolami et al.,[10] reported that the use of Gaio® in a mild to moderate hypercholesterolemic population previously subjected to a restricted diet intended to reduce their cholesterol was performed and results indicated a slight but statistically significant reduction in cholesterol content in subjects.

However, two long-term studies, which lasted 12 weeks and 6 months, respectively, failed to show any reduction in cholesterol levels in individuals who received Gaio yogurt, compared with that in control subjects at the end of treatment.[3,72]

Another probiotic product available in the market that contains an *E. faecium* strain is Walthers ECOFLOR (Walthers Health Care, Den Haag, The Netherlands). The producer claims the efficacy of the *E. faecium* strain against diarrhea, its anticarcinogenic effect, the production of enterocins active toward *L. monocytogenes*, the possible decrease of the LDL-cholesterol level, as well as its sensitivity to vancomycin and the production of L(+) lactic acid.

E. FAECIUM 18C23

A probiotic strain *E. faecium* 18C23 showed inhibited the adhesion of *E. coli* K88MB and *E. coli* K88ac to small intestinal mucus of piglets.[39,70] *E. faecium* WB2000 has expressed probiotic character by inhibiting cariogenic streptococcal biofilm formation.[84] *E. faecium* SE906 showed its ability to attach on the intestinal epithelium and barring pathogenic bacteria as *L. monocytogenes in vitro*. This strain was also safe to be used as it has low aminogenic potential, antibiotic resistance pattern and virulence determinants. It can be considered as a potential probiotic meat starter culture suitable for manufacture of dry-fermented Iberian sausages.[61]

11.4.1 DISADVANTAGES OF USING ENTEROCOCCUS SP.

The *Enterococcus* genus is not considered "generally recognized as safe, GRAS". Safety assessment for *enterococci* remains controversial. Even though harmless in healthy individuals, e*nterococci* are found to attack severe underlying diseases or impaired immune systems, or elderly people.[64] The rapid acquisition of antimicrobial resistance among *enterococci* probably contributes to their emergence as prominent nosocomial pathogens particularly the vancomycin-resistant enterococci have caused problems in hospitals on a global scale.[93] In particular, *E. faecium* clinical isolates are intrinsically resistant to high concentrations of penicillin.[40] About a dozen putative virulence factors have been reported from virulence analyses in various animal models.[86] Enterococci can cause food intoxication through production of biogenic amines in foods through decarboxylation of the

corresponding amino acids,[25] and care should be taken while selecting a probiotic strain belonging to *Enterococcus* group. Probiotic *E. faecium* strain is a possible recipient of the vanA gene cluster under *in vitro* conditions. Therefore, the risk for *in vivo* conjugation cannot be ruled out and should be considered in evaluations of the safety of *enterococcal* probiotics.[54]

11.5 *ESCHERICHIA COLI* NISSLESTRAIN 1917

This strain was originally isolated during World War I from a soldier who escaped a severe outbreak of diarrhea affecting his detachment from troops. NISSLE 1917, alias SK22, or DSM6601[63] seems to have a beneficial effect on several types of intestinal disorders and has been marketed as a probiotic since the early 1920s. *E. coli* strain NISSLE 1917 (O6:K5:H1) has been used as probiotic agent in medicine to treat chronic inflammatory and infectious diseases of the intestine.[5] *E. coli* NISSLE strain 1917 can be used for the treatment of patients with IBD.[65] *E. coli* NISSLE 1917 ameliorates colitis[77] via TLR-2- and TLR-4-dependent pathways in mice,[27] exhibited an inhibitory effect on the adhesion and invasion of intestinal epithelial cells by AIEC *in vitro,*[75] it stimulates immune response system and inhibit *Salmonella typhimurium in vivo.*[53] *E. coli* Nissle 1917 has capability to effect directly on the immune response mechanism by significantly stimulating specific humoral and cellular responses and simultaneously induces nonspecific natural immunity.[92] It can be used in treating chronic constipation without any side effects.[60] Guzy et al.[30] have elaborated that *E.coli* Nissles 1917 has profound effect on inflammatory diseases by regulating γδ T cell apotosis mediated via Toll-like receptor-2 by caspase- and FasLig and dependent pathways. Sun et al.,[82] have carried out work molecular level to understand the probiotic character of this strain and found that *E. coli* NISSLE 1917 meets all requirements and is recognized as a safe organism for human use. Despite its successful therapeutic application, only little is known about the beneficial traits contributing to the strains probiotic character. The mechanisms underlying the probiotic nature, especially at the molecular level, still have to be elucidated.[44]

11.6 CLOSTRIDIUM BUTYRICUM

Dr. Miyairi discovered C. *butyricum* MIYAIRI (CBM) from human faeces in 1933 at the department of Hygiene at Chiba Medical College (now located at

the Chiba University School of Medicine, Japan). *C. butyricum* has been used clinically to treat patients with intractable diarrhea and antibiotic-induced colitis in Japan, Korea, and China. It is stable against luminal digestion by gastric juices, and subsequently enters the colon, where it germinates, proliferates, and ultimately produces SCFA's. The mechanism by which CBM controls diarrhea is based on several properties like inhibiting putrefying bacteria and increase to beneficial bacteria (especially bifidobacteria and lactobacilli). CBM has been shown to have antagonistic interaction against *C. albicans*, *Clostridium difficile*, enterotoxigenic *E. coli*, *Klebsiella* sp., *Salmonella* sp., and *Vibriosp*.

C. butyricum MIYAIRI 588 strain has been used as a probiotic for both non-antimicrobial induced diarrhea and antimicrobial associated diarrhea in humans. Araki et al.,[5, 6] developed a yogurt based product containing the SCFA's derived from CBM and administered to mice and found that it reduced diarrhea. Cure of persistent infection with *H. pylori* in the gnotobiotic mice was demonstrated following infection with spores of *C. butyricum*.[41] Oral administration of CBM 588 alleviated DSS-induced colitis in rats as well as in humans. So CBM 588 administration may be done in place of butyrate enema.[66] The combined use of CBM and antibiotics reduced the changes in the intestinal flora and decreased the incidence of gastrointestinal side effects.[80] Mice administered only with vegetative cells of CBM showed reduction of H. *pylori* on the other hand *H. pylori* could not be detected in the mice administered with spores of CBM. The probiotic bacterium *C. butyricum* MIYAIRI strain 588 has preventive and therapeutic effects on EHEC O157:H7 infection in gnotobiotic mice.[85] The treatment period for CDAD reduced significantly when CBM with Vancomycin was given to patients.[24] *C. butyricum* MIYAIRI is effective for both the treatment and the prophylaxis of AAD in children, as it normalizes the intestinal flora disturbed by antibiotics.[78] Bio-Three drug containing *C. butyricum* was found effect immune response system in humans.[35] It also helps in better digestion of food along with free bowl movement. *C. butyricum* enhances humoral immune response in marine fish.[99]

11.7 BUTYRIVIBRIO FIBRISOLVENS

B. fibrisolvens is a commonly isolated bacterial species. The genus *Butyrivibrio* is found both in ruminal as well as cecal portions of the gastrointestinal tracts of mammals. *B. fibrisolvens* also resides in the intestine of humans, dogs, and cats but the numbers are usually low. It is a representative

of butyrate-producing ruminal bacterium with a high capacity to produce butyrate. The species has been described as a strictly anaerobic, curved, rod-shaped found single or in chains or filaments which may or may not be helical, which stains gram-negatively, but which apparently has a thin gram-positive cell wall, mol % G+C content of the DNA is 36–42(T_m).[13]

B. fibrisolvens administration as a probiotic showed suppression of chemically induced aberrant crypt foci (ACF), putative pre neoplastic lesions, in the rat colon and experimental colitis in rats.[6] It has the capability to isomerize the linoleic acid to conjugated linoleic acid (CLA) which has many health beneficial aspects such as preventing cancer and other diseases. This has also reduced β-glucuronidase activity in the contents of the colon and rectum.[65] Hence it can reduce risk of colon cancer.

11.8 YEASTS

S. boulardii, a species of yeast widely distributed, has been described as a biotherapeutic agent, since several clinical trials displayed its beneficial effects in the prevention and the treatment of intestinal infections and in the maintenance of inflammatory bowel disease. Experimental studies elucidated partially the molecular mechanisms triggered to improve the host health.[98] *S. boulardii* is one of the first and currently the only one commercialized yeast for human medicine.[58] *S. boulardii* survived the gastrointestinal tract's harsh conditions carried out using *in vitro* systems simulating human gastric/small intestinal (TIM) and large intestinal (ARCOL) environments.[21] In order to prevent the programmed cell death of *S. boulardii* S-adenosyl-L-methionine (AdoMet), may be taken along with the culture.[14]

Controlled clinical trials have shown that oral administration of *S. boulardii* could treat or prevent gastrointestinal diseases such as antibiotic-associated diarrhea, recurrent *C. difficile*-associated diseases, traveler's diarrhea, children acute diarrhea, enteral tube feeding-associated diarrhea, AIDS-associated diarrhea, rotavirus diarrhea intestinal bowel disease such as Crohn's disease and ulcerative colitis, and irritable bowel syndrome.[28] The protective effect against *C. difficile* has already been observed by many authors utilizing *S. boulardii.*[68] It also has beneficial effects such as promotion of iron absorption[55] and effective in preventing the recurrence of C. difficile-induced pseudo membranous colitis, as well as the antagonistic action of *E. coli.*[19] Yeasts can stimulate the immune response in fish.[26] *H. pylori* infected asymptomatic patients can be treated with triple therapy combined with *Lactobacillus GG*, *S. boulardii*, or *Lactobacillus/Bifidobacterium.*[41]

S. boulardii protease prevents *C. difficile* toxin A and B induced inhibition of protein synthesis in HT-29 cells.[15] *S. boulardii* prevents the degradation of intestinal layers from toxins and also stimulates the immune response.[2] The supplementation of trout (fish) starter diet with *S. boulardii* will be particularly useful for fast growing.[91] *S. boulardii* improved the electrolyte transport system in the pig jejunum.[76] Enteropathogenic *E. coli* mediated apoptosis of epithelial cells was delayed in the presence of *S. boulardii* and it exerted a protective effect on epithelial cells after EPEC adhesion by modulating the signaling pathway induced by bacterial infection.[19] Fermentation of soymilk with probiotic *S. boulardii* improves the biological functionality by making isoflavones biologically available, increased calcium content and easily digestible protein, reduction of glycosides and increasing in aglycones.[71]

Martins et al.[58] demonstrated that *S. cerevisiae* showed negligible adhesion ability; however, it could survive acidic conditions and grow in the presence of bile salts. *S. cerevisiae* 905, from "aguardente" (cachaça) production, showed viable intestinal populations with fecal levels which never fell below 10^6 cfu/g, and inhibited the proliferation of *S. typhimurium* in ileum and *C. difficile* in the colon and cecum of gnotobiotic mice. The ability to assimilate cholesterol *in vitro* and to tolerate low pH levels, gastric juice, and bile indicate that *S. cerevisiae* 832, and especially *S. cerevisiae* KK1 and *Isaatchenkia orientalis* KK5.Y.1 being more bile and gastric juice tolerant because of their human origin may be promising candidate strains for use as probiotics.[69]

Three strains of yeasts isolated from human gastrointestinal tract and feta cheese *viz., S. cerevisiae* 982*, S. boulardii* KK1 *and Kluyveromyces lactis* 630 strains exhibited increased polymorphonuclear cell influx and phagocytic activity as well as cytokine production in the air pouch which at par with potency as the probiotics Ultra levure *S. boulardii* and *L. acidophilus* NCFB 1748.[45]

K. lactis was most adhesive to Caco-2 cells. *K. lactis* proliferated under anaerobic conditions, and showed acid and bile tolerance; however, growth was moderate at 37 °C. Thus, it was concluded that *K. lactis* as a probiotic agent could be expected to enhance immune modulation rather than improve the intestinal flora.[47] The yeast strains namely *Pichia fermentans* BY5, *Pichia kudriavzevii* BY10, *P. kudriavzevii* BY15 and *Yarrowia lipolytica* HY4 isolated from raw milk showed probiotic characteristics like survival to gastric juices and adhesion to HT-29 cells and may serve as potential probiotics in assimilating cholesterol in the human intestine.[17]

KEYWORDS

- **antilisterial properties**
- **antimutagenic**
- ***Bacillus* species**
- ***E. coli***
- ***Enterococcus***
- **hypercholesterolemic**
- **immunomodulatory**
- **immunostimulatory**
- **probiotic**
- **probiotic *clostridium sp.***
- **probiotic yeast**
- **prophylactic**
- **enterotoxins**
- ***Weissella***

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PART IV
Food Laws, Acts, Orders, and Regulations

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CHAPTER 12

BRIEF HIGHLIGHTS AND IMPLICATIONS OF FOOD LAWS: CASE STUDY

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CONTENTS

ABSTRACT

Laws existed in a number of States in India for the prevention of adulteration of food- stuffs but they lacked uniformity having been passed at different times without mutual consultation between states. The International import/ export agencies of foods can refer to these food laws.

This chapter summarizes the details of food laws/acts/standards/orders and regulations that are mentioned in details in the official books by Government of India (GOI). Therefore, the reader is encouraged to refer to original guidelines by GOI. In 2006, all of the existing laws were consolidated to form one single statute in order to ensure systematic and scientific development of the food processing industry. The Food Safety and Standards Regulations (FSSR), 2011 notified in the Gazette of India came into force on August 5, 2011, to regulate manufacture, distribution and sale of nutraceuticals, functional foods, and dietary supplements in India. Food Safety and Standard Act (FSSA, 2006), FSSR (2010), Food Safety and Standards Rules (2011), and National Food Security Act (2013) are the very important and useful laws for providing safe and quality food to the consumers. These laws are flexible, modern, science based, and very clear.

12.1 INTRODUCTION

In recent years, food quality and safety have become an important issue in the public opinion, and media. Compelling evidence indicates inappropriate dietary habits as a leading cause of poor health that results in a worldwide increase in health care costs. These concerns make policymakers to declare a thematic priority to *Food Quality and Safety Programs*, *adulteration prevention*, *control over import, and export,* etc. in an attempt to improve the health and well-being of citizens through higher quality food and improved control over food production and related environmental factors.[4] There is need for identifying the major concerns for consumers along the food production chain, outlining issues associated with primary production, animal feeds, processing, distribution, consumption, and environmental health risks.[3,4]

12.1.1 SIGNIFICANT ROLE OF FOOD IN OUR LIFE

Food is the basic need of all living being for sustenance of life. All the religious and major faiths of the world have given top priority to food. Eating

is an important activity, which is required in order to live. It is no wonder to say that community health is national wealth. Right from the beginning of life, food has been the major source of energy and existence. A short review on food related laws, acts, rules and orders is valuable and advantageous. In India, there are number of pre-constitutional and post-constitutional laws, orders, rules, and acts that aim at the protection of the consumer interests with special reference to food quality, food safety, healthy food, and wholesome food. *The Supreme Court of India* has held that the right to life under Article 21 includes the right to a healthy and safe environment. Several acts and orders have prevailed in India to safeguard food safety and the health of the consumer. They were introduced to complement and supplement each other in achieving total food safety and quality. However, due to variation in the specifications/standards in different acts, and administration by different departments and ministries, there were implementation problems, and a lack of importance given to safety standards over a period of time.

Now a day there is lot advertisement of food and its items in television, newspaper, magazines, and on web sites. This shows that there is great market for food and there is increasing interest in general public about food and their health. The fresh, wholesome, healthy, of variety and in balanced diet food consumed can keep health very fit, and good. Food provides not only nutrients but it also provides minerals, macro and micronutrients, energy, and support. Food contains lot of microorganisms and when food is abused, miss-handled, under-processed, unhygienically handled and not given proper care in handling, packaging, transport and storage can cause food borne illness, or even death. There is more demand of food and food commodities due to much more increase in population but production of food is less due to more use of cultivating land for industries, homes, restaurant, railways, school, colleges, cinema halls, hospitals, etc. There are some food supplier and processing who want to earn more money by short-cut/illegal means by making adulteration in food items. This is extremely valuable to stop the mal-practices of food adulterations. To ensure safe, pure, and healthy food to public, government need to establish food laws, regulations, and acts. It does not make sense, from a scientific perspective, to allow processing and sale of potentially hazardous foods without regulation and inspection. This is especially true when there are well-documented cases of food—borne illness from consumption of foods that are non-hygienically produced, adulterated, and of low quality. We should base any changes to our food inspection laws on sound scientific evidence and credible data.[14,15]

This chapter includes certain Indian acts, which focus the right of consumer to approach the proper authority or adjudication for consumer's grievance to be sorted out, safety, and quality of the food is insured. In this chapter, authors have presented and discussed Indian food laws, codes, orders, acts, and regulations. They have also discussed implications of these laws.

12.2 INDIAN FOOD LAWS, CODES, ORDERS, ACTS, AND REGULATIONS

History of food laws, codes, orders, regulations, and acts in India is quite older. The Government of India (GOI) has passed many laws and regulation, from 1954 to 2013, related to food and its safety and to provide safe and healthy food to public. A list of major Indian food laws, orders, regulations, and acts are given below and briefly described in this chapter:

1. The Agricultural Produce (Grading and Marking) Act, 1937[1]
2. Prevention of Food Adulteration Act (PFA), 1954
3. Fruit Product Order (FPO), 1955
4. Export (Quality Control and Inspection) Act, 1963
5. Solvent Extracted Oil Control Order, 1967
6. The Insecticide Act, 1968
7. Rules (Ministry of Health and Family Welfare) with Last Amendments In, 1986
8. Bureau of Indian Standards (BIS) Act, 1986[2]
9. Environmental Protection and Pollution Control Act
10. Milk and Milk Products Order (MMPO), 1992
11. Infant Milk Substitutes Feeding Bottles and Infant Food (Regulation of Production, Supply) Act, 1992 and Rules, 1993
12. Food Product Order (FPO), 1995
13. Essential Commodities (EC) Act (Ministry of Food and Consumers Affairs), 1995[5]
14. Vegetable Oil Product Control Order, 1998
15. Food Safety and Standard Act (FSSA), 2006
16. Food Safety and Standards Regulations (FSSR), 2010
17. Food Safety and Standards Rules (FSSR), 2011
18. National Food Security Act (Right to Food Act), 2013

12.3 THE AGRICULTURAL PRODUCE (GRADING AND MARKING) ACT, 1937

The Agricultural Produce (Grading and Marking) Act, 1937 was given for the grading and marking of agricultural and allied commodities to make available only the quality agricultural produce to the consumers. As per this Act, the Central Government (CG) is authorized to make rules fixing grade designation to indicate the quality of any scheduled article, specifying grade designation, authorizing interested parties to grade, conditions regarding manner of marking packaging and providing for the confiscation, and disposal of produce marked in accordance with the provisions. AGMARK is a mark given under this Act.[1]

12.4 PREVENTION OF FOOD ADULTERATION (PFA) ACT, 1954

The Prevention of Food Adulteration Bill was passed by both the house of Parliament and received the assent of the President on September 29, 1954. It came into force on June 1, 1955 as *The Prevention of Food Adulteration Act, 1954 (37 of 1954).*[17]

India is in process of its developmental phage and updating and drafting time-to-time new food laws and regulation based on the requirements, and changes in global scenario. Food is one of the basic necessities for sustenance of life. Pure, fresh and healthy diet is most essential for the health of the people. In past, adulteration of foodstuffs was so rampant, widespread, and persistent that a comprehensive legislation became the need of the hour. To check this kind of anti-social evil a concerted and determined onslaught was launched by the Indian Government by introduction of the "*Prevention of Food Adulteration Bill-1954*" in the Parliament to ensure food safety and quality for the consumers at large.[17] Prevention of food adulteration act was established keeping the objectives in mind:

1. To protect the public from poisonous and harmful foods.
2. To prevent the sale of substandard foods.
3. To protect the interests of the consumers by eliminating fraudulent practices.

"Adulteration of food-stuffs and other goods" is now included in the Concurrent List (III) in the Constitution of India. It has, therefore, become possible for the Indian CG to enact all India legislation on this subject. This

act replaces all local food adulteration laws where they exist and also applies to those states where there are no local laws on the subject. Among others, it provides for:[17]

a. **Central Food Laboratory** Food samples can be referred for opinion in disputed cases.
b. **Central Committee** for Food Standards consisting of Central and State Governments (SG) to advise on matters arising from administration of the Act (Clause 3).
c. **Vesting in the CG** of the rule-making power regarding standards of quality for the articles of food and certain other matters (Clause 22).

12.4.1 PROHIBITION OF IMPORT OF CERTAIN ARTICLES OF FOOD

No person shall import into India: (a) any adulterated food; (b) any misbranded food; (c) any article of food for the import of which a license is prescribed; and (d) any article of food in contravention of any other provision of this Act or of any rule made thereunder.[17]

Application of Law Relating to Sea Customs and Powers of Customs Officers

1. The laws relating to sea customs and to goods, the import of which is prohibited by the Sea Customs Act, 1878, shall, subject to the provisions of Section 16 of this Act, apply in respect of articles of food, the import of which is prohibited under Section 5 of this act, and officers of customs and empowered under that act to perform the duties imposed thereby on a customs collector, and other officers of customs.
2. Without prejudice to the provisions of Sub-Section (1) the Customs Collector, or any officer of the Government authorized by the CG in this behalf, may detain any imported package, which is suspects to contain any article of food the import of which is prohibited under Section 5 of this Act and shall forthwith report such detention to the Director of the Central Food Laboratory and forward the send samples of any suspected article of food found therein to the said Laboratory.[17]

12.4.2 PROHIBITIONS OF MANUFACTURE, SALE OF CERTAIN ARTICLES OF FOOD

No person shall herself/himself by any person on his behalf manufacture, store, sell, or distribute:

1. Any adulterated food
2. Any misbranded food
3. Any article of food for the sale of which a license is prescribed
4. Any article of food in contravention of any other provision of this Act/any rule made
5. Any adulterant[17]

12.4.3 ANALYSIS OF FOOD

a. Public Analysts
b. Food Inspectors

12.5 FRUIT PRODUCTS ORDER (FPO), 1955

Fruit Products Order 1955, aims at regulating sanitary and hygienic conditions in manufacture of fruit, vegetable products. It is mandatory for all manufacturers of fruit, vegetable products to obtain a license under this Order.[6] To ensure good quality products, manufactured under hygienic conditions, the Fruit Product Order lays down the minimum requirements for:[11]

a. Sanitary and hygienic conditions of premises, surrounding and personnel
b. Water to be used for processing
c. Machinery and equipment
d. Product standards

12.6 EXPORT (QUALITY CONTROL AND INSPECTION) ACT, 1963

It was enacted by Parliament in 14th year of republic of India as follows: This Act may be called as Export (Quality Control and Inspection) Act 1963; it extends to the whole of India.

12.6.1 ESTABLISHMENT OF EXPORT INSPECTION COUNCIL

1. GOI by notification in the Official Gazette, establish with effect from such date as may be specified in the notification a Council to be known as the Export Inspection Council which shall consist of:

   a. Chairman to be appointed by the CG
   b. The Director of Inspection and Quality Control, ex-officio, who shall be the Secretary
   c. Honorary Adviser on Standardization and Director of Indian Standards Institution
   d. The Agricultural Marketing Adviser to the GOI
   e. The Director-General of Commercial Intelligence and Statistics
   f. The 15 members nominated by CG, three of whom agencies referred to in Section 7

2. The Council shall be a body corporate by the name aforesaid; having perpetual succession and a common seal, with power to acquire, hold and dispose of property and to contract, and shall by the said name sue and be sued.
3. No act or proceeding of the council shall be invalidated merely by reason of any vacancy in, or any defect in the constitution of the council.
4. Subject to such rules, the Council may appoint such officers and other employees, as it considers necessary for the purpose of discharging its functions under this Act.

12.6.2 DIRECTOR OF INSPECTION AND QUALITY CONTROL

The CG shall appoint a Director of Inspection and Quality Control to exercise powers and perform duties prescribed under this Act.

12.7 THE SOLVENT EXTRACTED OIL, DE-OILED MEAL, AND EDIBLE FLOUR (CONTROL) ORDER, 1967

This Order is basically a quality control order to ensure that the solvent extracted oils in particular are not reached to the consumers for consumption before the same are refined and conformed to the quality standards specified in the Order for the purpose. Standards for the solvent (hexane), which is to

be used for extraction of oil from the oil-bearing materials, have also been specified so as to eliminate possible contamination of oil from the solvent used.[3]

12.7.1 SALIENT FEATURES

- **a.** Governs the manufacture, quality, oils movement, de-oiled meal, and edible flour.
- **b.** Consumer protection, quality assurance of extracted oils, de-oiled meal, and edible flour.
- **c.** Eliminates the possibility of diversion of the oils for uses not intended.
- **d.** Prohibit buy, use, stock, solvent not conforming to the quality standards for extraction.
- **e.** Specifies particulars to be declared on the label affixed to the container.[3]

12.8 THE INSECTICIDE ACT, 1968

An Act to regulate the import, manufactures, sale, transport, distribution, and use of insecticides with a view to prevent risk to human beings, animals, and for matters connected therewith. Be it enacted by parliament in the 19th year of the republic of India as follows: Act may be called the Insecticide Act, 1968; extends to the whole of India; it came into force on date as the CG notified in the Official Gazette. The CG constituted *Central Insecticides Board* to advise the CG and SG on technical matters arising out of administration of this Act and to carry out the other functions. This law and its application are not directly involved in food processing therefore a very little information is provided in this chapter.[13]

12.9 BUREAU OF INDIAN STANDARDS ACT, 1986[2]

12.9.1 THE BUREAU OF INDIAN STANDARDS

1. With effect from the notification in the Official Gazette, there shall be established for the purposes of this Act, a Bureau, to be called the ***BIS.***

2. The Bureau shall be a body corporate, having perpetual succession and a common seal, with power, subject to the provisions of this Act, to acquire, hold and dispose of property, both movable and immovable, and to contract and shall by the said name sue and be sued.
3. The Bureau shall consist of the Minister-in-charge of the ministry or department of the CG having administrative control of the Bureau.[2]

12.10 ENVIRONMENTAL PROTECTION ACT, 1986

An Act to provide for the protection and improvement of environment and for matters connected there with: To take appropriate steps for the protection and improvement of human environment; and whereas it is considered necessary to implement the decisions aforesaid in so far as they relate to the protection and improvement of environment and the prevention of hazards to human beings, other living creatures, plants, and property; be it enacted by Parliament in the 37th year of the republic of India as follows: (a) This Act may be called as the *Environment (Protection) Act, 1986*. (b) It extends to the whole of India.

12.11 CONSUMER PROTECTION ACT, 1986

The Consumer Protection Act, 1986 is a milestone in the history of socio–economic legislation in India. It aims at providing an informal, inexpensive and expeditious justice to the consumers aggrieved by defects in goods, or deficiency in services. An Act to provide for the better protection of the interest of consumers and for that purpose to make provision for the establishment of consumer councils and other authorities for the settlement of consumers' disputes and for matters connected therewith.

12.12 POLLUTION CONTROL ACTS

Following are the list of laws made under and amended under this department:

a. The Water (Prevention and Control of Pollution) Act, 1974
b. The Water (Prevention and Control of Pollution) Rules, 1975
c. The Central Board for the Prevention and Control of Water Pollution Rules, 1975

d. The Water (Prevention and Control of Pollution) CESS Act, 1977
e. The Water (Prevention and Control of Pollution) CESS Rules, 1978
f. The Air (Prevention and Control of Pollution) Act, 1981
g. He Air (Prevention and Control of Pollution) Rules, 1982
h. Air (Prevention and Control of Pollution) (Union Territories) Rules, 1983
i. The Environment (Protection) Act, 1986
j. The Environment (Protection) Rules, 1986
k. Coastal Regulation Zone (CRZ)
l. Environment Impact Assessment Notification, 2006
m. The Hazardous Wastes Rules, 2008
n. Chemical Accidents (Emergency Planning, Preparedness, Response) Rules1996
o. The Bio-Medical Waste (Management and Handling) Rules, 1998
p. The Plastics Manufacture, Sale and Usage Rules, 1999
q. The Municipal Solid Wastes (Management and Handling) Rules, 2000
r. The Noise Pollution (Regulation and Control) Rules, 2000
s. The Ozone Depleting Substances (Regulation and Control) Rules, 2000
t. The Batteries (Management and Handling) Rules, 2001
u. The Central Motor Vehicles Rules, 1999
v. The Public Liability Insurance Act, 1991
w. The National Environment Tribunal Act, 1995

Because these regulations are not directly involved in food, processing, therefore full details are omitted in this chapter.

12.13 MILK AND MILK PRODUCTS ORDER, 1992

MMPO 1992 administered by the Department of Animal Husbandry and Dairying under Ministry of Agriculture was promulgated on June 9, 1992 under the provision of Section (3) of the *EC Act 1955* with a view to maintain an increased supply of liquid milk of desired quality to the general public. This order regulated production, supply and distribution of milk, and milk products throughout the country. The order also seeks to ensure the observance of sanitary requirements for dairies, machinery and premises, and quality control standards for milk, and milk products.[6,15]

12.14 INFANT MILK SUBSTITUTES FEEDING BOTTLES AND INFANT FOOD (REGULATION OF PRODUCTION, SUPPLY) ACT, 1992 AND RULES, 1993

This is an Act to provide the regulation of production, supply and distribution of infant milk substitutes, feeding bottles, and infant foods with a view to the protection and promotion of breastfeeding and ensuring the proper use of infant foods and for matters connected therewith or incidental thereto. It enacted by Parliament in the 43rd year of the republic of India as *"The Infant Milk Substitutes, Feeding Bottles and Infant Foods (Regulation of Production, Supply and Distribution) Act, 1992"* and it extends to the whole of India.[12]

12.15 FOOD PRODUCT ORDER, 1995

The Fruit Products Order 1955, promulgated under Section 3 of the *EC Act 1955*, with an objective to manufacture fruit and vegetable products maintaining sanitary and hygienic conditions in the premises and quality standards laid down in the Order.[6,7]

12.16 ESSENTIAL COMMODITIES (EC) ACT, 1995

The EC Act 1995 gives powers to control production, supply, and distribution of EC for maintaining or increasing supplies and for securing their equitable distribution and availability at fair prices. The EC Act is being implemented by the SG/UT Administrations by availing of the delegated powers under the Act. The SG/UT Administrations have issued various control orders to regulate various aspects of trading in EC such as food grains, edible oils, pulses kerosene, sugar, etc. The CG regularly monitors the action taken by SG/UT Administrations to implement the provisions of the EC Act, 1995.[3,6]

12.17 VEGETABLE OIL PRODUCTS ORDER, 1998

The Vegetable Oil Products industry is regulated by Vegetable Oil Products Order 1998 through the Directorate of Vanaspati, Vegetable Oils, and Fats, Department of Food, Public Distribution, Ministry of Consumer Affairs, and Food and Public Distribution. The Vegetable Oil Products Order, 1947 and Vegetable Oil Products (Standards of Quality) Order, 1975 have been

replaced by a single Order called "Vegetable Oil Products (Regulation) Order, 1998" for proper regulation of manufacture, distribution, and sale of Vegetable Oil Products. Salient features of the order are:[21,22]

a. The procedure of registration has been simplified.
b. The standards of quality prescribed under the schedule have been tightened.
c. The vogue and non-measurable arbitrary interpretation have been amended.
d. Consumers' protection through quality assured.

12.18 FOOD SAFETY AND STANDARDS ACT (FSSA), 2006

The FSSA 2006 is an act to consolidate the laws relating to food and to establish the *Food Safety and Standards Authority of India (FSSAI)* for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food for human consumption, and for matters connected therewith or incidental thereto.[8]

12.18.1 ESTABLISHMENT OF FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA8

1. The CG shall establish a body to be known as the ***FSSAI*** to exercise the powers conferred on, and to perform the functions assigned to, it under this Act.
2. The head office of the Food Authority shall be at Delhi.
4. The Food Authority may establish its offices at any other place in India.

12.18.2 REASONS FOR EXISTENCE OF FSSA, 2006[8]

- There was multiplicity of food laws, standards setting, and enforcement agencies for different sectors of food.
- Varied quality/safety standards restricting innovation in food products.
- Lack of manpower, poor laboratories infrastructure, and other resources non-conducive to effective fixation of standards.

- Standards rigid and non-responsive to scientific advancements and modernization.
- Poor information dissemination to consumer level.

12.18.3 SALIENT FEATURES OF FSSA, 2006[8]

- Unified licensing procedures -Single Window
- Common application forms and procedures for licensing procedures
- Distinction between "registration" and "licensing"
- Cut off limits for registration and licensing
- Two tier system of licensing: Central licensing system and State licensing system
- Introduction of exhaustive safety, sanitary, and hygienic conditions mandatory for registration/licensing
- Less inspections, more audit of system
- Time limit of 60 days for processing of license
- Thrust on Preventive Actions
- Big manufacturing units under central licensing FSSAI (Delhi)
- Pre-Inspection compulsory before giving license
- Provision of improvement notices
- Provision of annual returns before 31st May
- Fine/Penalty through adjudication
- Punishment through court
- Special import regulation

This act consists of 101 sections and 2 schedules. The Second Schedule gives the details of Food Act/Orders, which will stand repealed on commencement of the Section 97 of the Act. Those were:[8]

a. The Prevention of Food Adulteration Act 1954 (37 of 1954).
b. The Fruit Products Order 1955.
c. The Meat Food Products Order 1973.
d. The Vegetable Oil Products (Control) Order 1947.[22]
e. The Edible Oils Packaging (Regulation) Order 1998.
f. The Solvent Extracted Oil, De oiled Meal, and Edible Flour (Control) Order 1967.
g. The MMPO 1992.
h. Any other order issued under the EC.

12.19 FOOD SAFETY AND STANDARDS REGULATIONS (FSSR), 2010

12.19.1 REGISTRATION AND LICENSE FOR FOOD BUSINESS

All Food Business Operators (FBO) in the country will be registered or licensed in accordance with the procedures without prejudice to the availability of safe and wholesome food for human consumption.[9]

12.19.2 REGISTRATION OF PETTY FOOD BUSINESS (PFB)

1. Every PFB shall register with Registering Authority by submitting registration application along with a fee.
2. The FBO shall follow the basic hygiene and safety requirements provided in Schedule 4 (Part I) of these Regulations and provide a self—attested declaration of adherence to these requirements with the application under Schedule 2.[9]

12.19.3 LICENSE FOR FOOD BUSINESS (FB)

No person shall commence any FB unless he possesses a valid license under these Regulations.[9]

12.19.3.1 MODIFICATIONS, EXPANSION OR CHANGES IN PREMISE(S) AFTER GRANT OF LICENSE OR REGISTRATION

FBO shall ensure that the Registering or Licensing Authority always has up-to-date information on their FB establishments.[9]

12.19.3.2 TRANSFER OF REGISTRATION CERTIFICATE OR LICENSE IN CASE OF DEATH

(1) In the event of death of the holder of a Registration certificate/license, such certificate/license shall subsist for the benefit of the legal representative/family member of the deceased, or until the expiry.[9]

Part I: General Hygienic and Sanitary Practices to Be Followed by PFB Operators Applying for Registration

A. Sanitary and Hygienic Requirements for Food Manufacturer/Processor

The place where food products are manufactured, shall comply with all the requirements asked in this law.

Part II: Requirements on Hygienic and Sanitary Practices to be Followed by all FBO

The establishment in which food is being handled, processed, manufactured, packed, stored, and distributed by the FBO and persons handling them should conform to the sanitary and hygienic requirement, food safety measures, and other standard as specified below. It shall also be deemed to be the responsibility of the FBO to ensure adherence to necessary requirements. In addition to the requirements specified below, the FB shall identify steps in the activities of FB, which are critical to ensuring food safety, and ensure that safety procedures are identified, implemented, maintained, and reviewed periodically.[9]

Location and Surroundings
Equipment
Facilities

a. Water supply
b. For cleaning utensils/equipments
c. Washing of raw materials
d. Ice and steam
e. Drainage and waste disposal
f. Personnel facilities and toilets
g. Air quality and ventilation
h. Lighting

Food Processing/Preparation, Packaging, and Distribution/Service Food Packaging

Packaging materials shall provide protection for all food products to prevent contamination, damage and shall accommodate required labeling as laid down under the FSS Act, and the Regulations there under.[9]

Management and Supervision

A detailed Standard Operating Procedure (SOP) to be developed for proper management, which in turn would help in identifying any problem at exact point, so the course of damage control would be faster.[9]

Food Testing Facilities
Audit, Documentation and Records
Sanitation and Maintenance of Establishment Premises
Personal Hygiene

Health Status:
Visitors:

Product Information and Consumer Awareness
Training

I. Good Manufacturing Practices for Whole Premises

Food Preparation Areas: There will be no smoke nuisance in the food preparation area. Wherever cooking or frying of any kind is being done, a chimney having appropriate suction capacity as per the size of the kitchen has to be installed prior to start of business.

Hand washing facilities and toilets: (a) Adequate number of wash-hand basins shall be provided to wash hands, with hot and cold running water, and materials for cleaning hands, and drying them hygienically. (b) Separate sinks must be provided for washing raw food and cleaning equipment. (c) Sinks with a draining board, detergent, and hot water shall be provided to ensure proper cleaning of utensils, crockery and cutlery, a separate place for washing pots, and pains. (d) There must also be enough toilets and those must not lead directly into food areas.

Changing Facilities: Facilities for staff to change their clothes must be provided.[9]

II. Good Food Hygiene Practices

Cleaning
Cleaning instructions
Raw materials
Preparation of fruits/vegetables

Cooking
Chilling
Cross-contamination

III. Personal Hygiene

IV. Transportation and Handling of Food

V. Storage

(1) It is very important to store food properly for the purpose of food safety.

Stock Rotation: The rule is FIFO (first in, first out) to make sure that older food is used first. This will help to prevent wastage.

VI. Special Requirements for High Risk Foods

This section deals selectively with few varieties of food, which are high risk as per HACCP and may need special attention. The type of foods covered here are as follows:

a. Cut fruits/salads, fresh juices, and beverages.
b. Confectionery products.
c. Milk and dairy products.
d. Water based chutneys, sauces, etc.
e. Foods transported to point of sale from the point of cooking.
f. Foods with gravy.
g. Fried foods.
h. Proper quality/branded oils/fats should be used for food preparation, frying, etc.
i. Post cooked mixing.
j. Ingredients added to the cooked food should be thoroughly cleaned.
k. Thawing of Frozen Products (a) Frozen products should be thawed in refrigerator/microwave/convection oven or under running potable water well before cooking, (b) Only required portion of the food should be thawed at a time, and (c) Thawed products should be used immediately and not refrozen or kept in chiller.[9]

Packaging: General Requirements (Regulation 4.1.1)
Labeling

General Labeling Requirements

Labeling of Pre-packaged Foods:

The Name of Food

12.20 FOOD SAFETY AND STANDARDS REGULATIONS (FSSR) 2011

These regulations may be called the FSSR (Prohibition and Restrictions on sales) 2011. In addition to the functions entrusted to it under the Act, the Referral Laboratory shall carry out the following functions:[10]

a. Analysis of samples of food sent by any officer/authority authorized by the Food Authority for the purpose and submission of the certificate of analysis.
b. Investigation for the purpose of fixation of standard of any article of food.
c. Investigation in collaboration with the laboratories of food analysts in the various States for the purpose of standardizing methods of analysis.
d. Ensuring that the laboratory follows the scientific protocols laid down for handling/testing the articles of food.
e. Maintaining high standards of accuracy, reliability, and credibility in operation of laboratory, achieving, and maintaining required levels of accreditation and reliability.
f. Laying down mechanism for ensuring that personnel of the laboratory adhere to high professional standards and discipline.
g. Such other conditions, as the Authority may lay down for Referral Laboratories.
h. Capacity building by organizing professional training, workshops and seminars for the Food analyst, laboratory personnel in the states specified by the Food authority.

Restriction on use of certain ingredient

Prohibition on sale of food articles coated with mineral oil

Food Products Standards and Food Additives regulations 2011

Restriction on sale of Carbia Callosa and Honeydew:
Product not to contain any substance, which may be injurious to health:
Restriction on sale of Kangra tea:
Condition for sale of flavored tea:
Restriction on sale of common salt:
Use of flesh of naturally dead animals or fowls prohibited:

12.20.1 REGULATORY REQUIREMENTS FOR ENTRY IN INDIA

As the nutraceutical regulation is evolving in India, with the recent implementation of FSSA, there is a possibility that some of the content is conflicting/confusing, but for Indian industry to take a shape, this has to be streamlined. In order to enter the Indian nutraceutical market, some of the very important areas to focus include product evaluation, actual product analysis, procuring licenses, and developing India-specific health and label claims.[10,18]

12.21 THE NATIONAL FOOD SECURITY ACT, 2013

The **National Food Security Act 2013** (**Right to Food Act**) is an Act of the Parliament of India, and it extends to whole of India. It aims to provide for food and nutritional security in human life cycle approach, by ensuring access to adequate quantity of quality food at affordable prices to people to live a life with dignity and for matters connected therewith or incidental thereto. It was signed into law on September 10, 2013, retroactive to July 5, 2013.The National Food Security Act, 2013, converts into legal entitlements for existing food security programs of the GOI.[16]

Food Security Allowance

In case of non-supply of the entitled quantities of food grains or meals to entitled persons under as per the guidelines of "*The National Food Security Act, 2013*", such persons shall be entitled to receive such food security allowance from the concerned SG to be paid to each person, within such time and manner as may be prescribed by the CG.[16]

Identification of Eligible Households
Reforms in Targeted Public Distribution System
Women Empowerment
Grievance Redressal Mechanism
Transparency and Accountability
Provisions for Advancing Food Security

12.22 CERTIFICATION MARKS IN INDIA

India has a comprehensive system of product certifications governed by laws made by the **Parliament of India** at various times. These certifications

are managed by various agencies, and hold various statuses before the law. Some of these marks are mandatory for the products to be manufactured or to be placed in the Indian market while some of the marks hold only an advisory status. All the industrial standardization and industrial product certifications are governed by ***The BIS***, ***The national standards organization of India***, while standards for other areas (like agricultural products) are developed and managed by other governmental agencies. The state enforced certification marks presently in India are:

- **AGMARK:** This mark is necessary for all agricultural products. Affixing or printing AGMARK on a food product means that the particular food item satisfies the standard prescribed under the Act. It provides assurance of quality to the consumers.
- **BIS Hallmark:** These marks certify the purity of gold jewelry.
- **Ecomark:** Ecolabel on various products issued by *BIS* Voluntary and promotional.
- **FPO Mark:** A mandatory mark for all processed fruit products in India. Certifies that the product was manufactured in a hygienic "food-safe" environment.
- **ISI Mark:** For industrial product, ISI certifies that a product conforms to a set of standards laid by the *BIS*.
- **The India Organic:** These marks are certification mark for organically farmed food products. And certifies that the product conforms to the specifications of *National Standards for Organic Products, 2000* and any eventual amendments.
- **The Non-Polluting Vehicle Mark:** This mark on motor vehicles certifies conformity to the **Bharat stage emission standards.**

12.22.1 OTHER MARKS

These are mandatory marks or labels required by the law in India, but are not exactly certifications marks.

- **Brown Dot Symbol:** This mark shows that the product is Non-vegetarian. Either of this is mandatory for packaged food products. To distinguish between vegetarian and non-vegetarian food.
- **Green Dot Symbol:** This mark shows that the product is vegetarian.
- **Toxicity Label**: Mandatory on the containers of pesticides sold in India. Identifies the level of toxicity of the pesticide in four levels.

12.22.2 NON-STATUTORY MARKS

There are other non-statutory certification marks or schemes in India, which are promoted by the GOI, by policy, or through governmental or semi-governmental agencies.

- **Silk Mark:** Certifies that a piece of textile is pure silk. Managed by the *"Silk Mark Organization of India.*"
- The **Ayush Mark or the Ayush Product Certification Scheme** for herbal products by the *Department of Ayush*.
- **The Darjeeling Tea Certification Mark** is a geographical indication mark for tea produced in Darjeeling.

12.23 FOOD LAWS MAKING BODIES AND SUPPORTING ORGANIZATIONS: INDIA

1. FSSAI, Ministry of Health and Family Welfare, GOI.
2. Indian Institute of Public Health (IIPH), Hyderabad.
3. Bureau of Indian Standards (BIS).
4. Quality Council of India (QCI).
5. Ministry of Health and Family Welfare, GOI.
6. Ministry of Consumer Affairs, Food and Public Distribution, GOI.
7. Agricultural and Processed Food Products Export Development Authority.

12.24 FOOD ANALYSIS LABORATORY IN INDIA

a. Central Food Laboratory, Kolkata
b. Central Food Laboratory, Ghaziabad
c. Central Food Laboratory, Mysore
d. Central Food Laboratory, Pune

These laboratories are also known as the referral laboratories given by the "FSSAI."

12.25 CONCLSIONS

There is need to clarify and formulate the regulatory framework. If substantiation effectively enforced the FSSA, there is the potential to open up tremendous opportunity for the novel, innovative, functional food, and nutraceutical industry.[20] There are certain recommendations that could bring about improvements in existing regulations such as: to grab a larger pie from this world opportunity, Indian producers of products should unite to form a platform to market India as a brand. There is a need for an increased collaboration on the manufacturing and research and development front among Indian manufacturers. There has to be coordination among all agencies, including policy makers, regulators, and manufacturers. The manufacturing, validation, research and development, and intellectual property protection needs to be standardized. There should be expansion of Indian standards like Indian Pharmacopoeia[19,20] complies with their safety and quality standards. Indian government has still to amend its laws regarding nutrition labeling so that consumers become aware of safe and healthy facts of foods. Joint efforts by the government and private agencies in terms of suitable legislation and help from food scientists show there is tremendous potential for processed food in India in the future. To conclude, the passing of the food laws 2006, 2010, 2011, and 2013, are significant steps but a lot more has to happen to eliminate the overlap of old laws and regulations. Prior to the FSSA, there were multiple laws and regulations governing food safety and standards.

KEYWORDS

- **acts**
- **adulteration**
- **agriculture**
- **amendments**
- **article**
- **bureau**
- **consumer**
- **control**
- **court**
- **essential commodities**

- **export**
- **food laws**
- **food regulations**
- **food safety**
- **fruit product**
- **gazette**
- **Government of India (GOI)**
- **Indian laws**
- **Indian standards**
- **infant food**
- **inspection**
- **laws**
- **marking**
- **ministries**
- **notification**
- **orders**
- **penalty**
- **preservation**
- **prevention**
- **production**
- **right**
- **rules**
- **safeguard**
- **scientific**
- **standards**
- **vegetable oil**

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APPENDIX I GLOSSARY OF TERMS

Adulterant: Any material, which is or could be employed for the purposes of adulteration.

Anganwadi: A childcare and development center set up under the *Integrated Child Development Services Scheme* of the CG to render services covered under Section 4, clause (a) of sub-Section (1) of Section 5 and Section 6.

Central Food Laboratory: Means any laboratory or institute established or specified under Section made there under.

Central Pool: The stock of food grains which is: (a) procured by the CG and the SG through minimum support price operations; (b) maintained for allocations under the TPDS, other welfare schemes, including calamity relief, and such other schemes; (c) kept as reserves for schemes referred to in sub-clause (b).

Eligible Households: Households covered under the priority households and the *Antyodaya Anna Yojana* referred to in S-Section (1) of Section 3.

Fair Price Shop: A shop, which has been licensed to distribute essential commodities by an order issued under Section 3 of the *EC Act*, 1955, to the ration cardholders under the TPDS.

Feeding Bottle: Bottle/receptacle used for the purpose of feeding infant milk substitutes and includes a teat, and a valve attached or capable of being attached to such bottle or receptacle.

Food Safety Plan (FSP): FSP are scheme, program, methods identifying responsibilities, action areas, and worked out for achieving objective of food safety in a locality, such as panchayat, taluka, municipality, and district. FSP is supposed to involve the public in food safety program from village, town community, local bodies, panchayats, and other stakeholders.

Food Additives: Any substance used as a typical ingredient of the food and intentionally added to food for a technological purpose in the manufacture, processing, preparation, treatment, packing, packaging, and transport.

Food Grains: Rice, wheat or coarse grains, or any combination thereof conforming to such quality norms as may be determined, by order, by the CG from time-to-time.

Food Security Allowance: The amount of money to be paid by the concerned SG to the entitled persons under Section 8.

Food Security: The supply of the entitled quantity of food grains and meal specified.

Functional Foods: Foods that encompass potentially healthful products including any modified food or ingredient that may provide a health benefit

beyond the traditional nutrients it contains. In India, FSSAI is working on developing regulations for functional foods.

Health Care System: An institution or organization engaged, either directly or indirectly, in health care for mothers, infants, or pregnant women and includes a health worker in private practice, but does not include a pharmacy or drug store.

Health Worker: A person engaged in health care for mothers, infants, or pregnant women.

Import: Bringing into any place within the territories, to which this Act extends from a place outside those territories.

Infant Food: Any food being marketed or otherwise represented as a complement to breast milk to meet the growing nutritional needs of the infant after the age of four months.

Infant Milk Substitute: Any food being marketed or otherwise represented as a partial or total replacement for mother's milk, whether or not it is suitable for such replacement.

Label: Any written, printed, or graphic matter on the immediate package and on every other covering in which the package is placed or packed and includes any written, printed, or graphics matter accompanying.

Label: Display of written, marked, stamped, printed, graphic matter affixed, or appearing upon, any container.

Local (Health) Authority: In relation to a local area, the officer appointed by the CG or SG, by notification in the Official Gazette, to be in-charge of health administration in such area with such designation as may be specified therein.

Local Area: Any area, whether urban or rural, declared by the CG or SG by notification in the Official Gazette, to be a local area for the purposes of Act.

Manufacture: Any process incidental or ancillary to the manufacture of an article of food.

Meal: Hot cooked or pre-cooked and heated before its service meal or take home ration, as may be prescribed by the CG.

Minimum Support Price: The assured price announced by the CG at which food grains are procured from farmers by the CG and the SG and their agencies, for the central pool.

Notification: A notification issued under this Act and published in the Official Gazette.

Other Welfare Schemes: Government schemes, in addition to the TPDS, under which food grains or meals are supplied as part of the schemes.

Package: A box, bottle, casket, tin, barrel, case, receptacle, sack, bag, wrapper, or other thing, in which an article of food is placed or packed.

Person with Disability: A person defined as such in clause (*t*) of Section 2 of the Persons with Disabilities (Equal Opportunities, Protection of Rights, and Full Participation) Act, 1995.

Premises: Shop, stall, place where any article of food is sold, manufactured, or stored for sale.

Primary Food: Article of food, being a producer of agriculture/horticulture in its natural form.

Priority Households: Households identified as such under Section 10.

Ration Card: A document issued under an order or authority of the SG for the purchase of essential commodities from the fair price shops under the TPDS.

Rural Area: Any area in a state except those areas covered by any urban local body or a cantonment board established or constituted under any law for the time being in force.

Sample: A sample of any article of food taken under the provisions of stated Act.

Senior Citizen: A person defined as such under clause (*h*) of Section 2 of the Maintenance and Welfare of Parents and Senior Citizens Act, 2007.

Social Audit: The process in which people collectively monitor and evaluate the planning and implementation of a program or scheme.

Targeted Public Distribution System (TPDS)**:** The system for distribution of essential commodities to the ration card holders through fair price shops.

Trans Fatty Acids (TFAs): These are primarily associated with Vanaspati during the process of partial hydrogenation that affects is a significant component of Indian diet. TFAs links with diabetes and cardiovascular diseases (CVDs) is well established. Keeping in view the risks involved, FSSAI proposes to come out with regulatory standards for TFAs.

Unwholesome/Noxious: When used in relation to an article of food mean respectively that the article is harmful to health or repugnant to human use.

Vigilance Committee: A committee constituted under Section 29 to supervise the implementation of all schemes under this Act.

Worker: A person employed under a contract of service or apprenticeship, such as on garden, grounds and out-houses, and for any fittings.

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CHAPTER 13

FOOD REGULATIONS AROUND THE GLOBE

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CONTENTS

ABSTRACT

Food spoilage is any undesirable change that renders a product unsafe for human consumption. Food spoilage causes enormous financial losses and is roughly estimated that almost 35% of the farm produce is spoiled due to several reasons. It is estimated that quarter of the world's food commodities are lost through microbial activity alone. Spoilage of foods is a frequent problem, which impacts health, economy, and food security. To safeguard the food products and ensure public health several agencies are imposing food regulations including FSSAI, USFDA, CFIA, and others. The major objective of implementing regulation is to cater the needs of the consumers related to safe and wholesome food and food products.

13.1 INTRODUCTION

Food is defined as a "*substance, whether processed, partially processed or unprocessed, which is intended for human consumption and includes primary food, genetically modified or engineered food, infant food, packaged drinking water, alcoholic drink, chewing gum, and any substance, including water used into its preparation or treatment.*"[7]

A food supply chain or food system is referred to as "the processes that describe how food from a farm ends up on our tables". The process includes production, processing, distribution, consumption, and disposal. Food moves systematically in domino-like motion from producers to consumers, while the money consumers pay for food goes to people who work at various stages along the food supply chain in the reverse direction. When one part of the food supply chain is affected, the whole food supply chain is affected, which is often manifested through changes in price.

But apart from the price factor, the effect is also visible in the food safety domain. Since, there are so many stakeholders in the chain from farm to fork; this makes it more essential and difficult to manage the safety of food. The chances of food getting unsafe or below quality are high. Thus, to monitor and regulate this issue of food safety during manufacturing, processing, distribution, sale, and import we need an authority that states a set of standards and guidelines, in relation to food articles. This will ensure availability of only safe and wholesome food to the consumers. A few of such regulatory bodies are:

a. Food Safety and Standards Authority of India (FSSAI), India;
b. European Food Safety Authority (EFSA), European Union;
c. United States Food and Drug Administration (USFDA), USA;
d. Canadian Food Inspection Agency (CFIA), Canada;
e. Bundesanstaltfür Landwirtschaft und Ernährung, Germany;
f. New Zealand Food Safety Authority (NZFSA), New Zealand;
g. Australian Quarantine and Inspection Service, Australia;
h. Food Standards Australia New Zealand (FSANZ);
i. Advisory committee on the microbiological safety of food (ACMSF);
j. Norwegian Ministry of Agriculture and Food, Landbruks-ogmatdepartementet;
k. Bundesamtfür Verbraucherschutz und Lebensmittelsicherheit.

13.2 FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA (FSSAI)

The Food Safety and Standards Authority of India, established under Food Safety and Standards Act, 2006, had laid down science based standards for food products and measures to regulate manufacturing, storage, distribution, sale and import of food products to ensure availability of safe and wholesome food.

13.2.1 HISTORY AND GOALS

13.2.1.1 FOOD SAFETY AND STANDARD BILL, 2005

The Act came into existence due to a constant need for comprehensive legislation on Food with a Food Regulatory Authority concerning both domestic and export markets. In 1998, need for the bill was avowed by the Subject Group on Food and Agro Industries, followed by Parliamentary Committee on Pesticide Residues in 2004 and later in 2005 by the Standing Committee on Agriculture.

The Food Safety and Standard Bill were drafted with a purpose to eliminate multi-level and multi-departmental controls. Under the new Bill, all of the previously existing bills were consolidated and compiled, which included:

a. Edible Oils Packaging (Regulation) Order (1988),
b. Fruit Products Order (1955),

c. Meat Food Products Order (1973),
d. Milk and Milk Products Order (1992),
e. Prevention of Food Adulteration Act (1954),
f. Solvent Extracted Oil, De-Oiled Meal and Edible Flour (Control) Order (1967), and
g. Vegetable Oil Products (Control) Order (1947).

After exhaustive discussions, the draft Bill was approved by the Group of Ministers constituted by the Government of India (GOI) and was coined as "***The Food Safety and standards Bill, 2005***".

13.2.1.2 FOOD SAFETY AND STANDARD ACT, 2006

The Bill was presented in Parliament, and after passing by both the Houses, It received the assent of President on August 23, 2006. The FSSAI aims to establish a single reference point for all matters relating to food safety and standards, by moving from multi-level, multi-departmental controls to a single line of command and hence established an independent statutory Authority with head office at Delhi.[7]

13.2.1.3 ESTABLISHMENT OF THE AUTHORITY

Implementation of FSSAI is under the administrative control of Ministry of *Health and Family Welfare, Government of India*. The Chairperson (rank of Secretary, GOI) and Chief Executive Officer of FSSAI are appointed by Government of India. FSSAI performs following functions:

- To lay down science based standards.
- To install comprehensive Food Monitoring Systems.
- To ensure safe, hygienic and wholesome food is produced by food manufacturers and distributed by food operators.
- To harmonize the National Standards with International Standards (Codex).
- To regulate Import/Export of safe foods.
- To lay down mechanisms and guidelines for accreditation of certification bodies.
- To lay down procedure and guidelines for accreditation and notification of laboratories.

- To collect and collate data regarding food consumption, incidence and prevalence of biological risk, contaminants in food, residues of various, contaminants in foods products, identification of emerging risks and introduction of rapid alert system.
- To create an information network so that the public, consumers, Panchayats, and others do receive a rapid, reliable, and objective information about food safety and issues of concern.
- To provide training programs for entrepreneurs or upcoming entrepreneurs in food sector.

13.2.1.4 STRUCTURE OF FOOD SAFETY AND STANDARDS AUTHORITY

The members of Authority consists of Chief Executive Officer (Chairperson) and representatives from: Ministry of Health and Family Welfare, Ministry of Agriculture, Ministry of Commerce, Ministry of Consumer Affairs, Ministry of Food Processing Industries, Ministry of Law and Justice, Ministry of Micro, Small and Medium Enterprises, two representatives respectively from food Industry/ farmers organization and from consumer organization, three eminent food technologists/scientists, five representatives from States, and one representative from Retailer's organizations.

The FSSAI works on the advice of the Central Advisory Committee, scientific committee and various scientific panels. Scientific panels of FSSAI are comprised: for functional foods, nutraceuticals, dietetic products and other similar products; method of sampling and analysis; food additives, flavorings, processing aids and materials in contact with food; contaminants in the food chain; biological hazards; pesticides and antibiotic residues; labeling and claims/advertisements; genetically modified organisms and foods and fish and fisheries products.[8]

13.2.2 FOOD SAFETY AND STANDARD ACT, 2006

The FSSAI came into existence on August 23, 2006 and consists of 12 chapters in total, comprising of 101 sections and 2 Schedules. The main features of the Act include:

a. Movement from multi-level and multi-departmental control to integrated line of command;

b. Licensing for manufacture of food products, which was granted by the Central Agencies under various Acts and Orders, would stand decentralized to the Commissioner of Food Safety and his officer;
c. Single reference point for all matters relating to Food Safety and Standards, regulations and enforcement and shift from mere regulatory regime to self-compliance through Food Safety Management Systems.

The FSSAI Act gives a brief introduction and definitions for general terms used throughout the Act such as adulterant, food, advertisements, claim, etc. The Act also lays down the clauses on procedures of selection of different Committees, Chairperson and other members, their term of service, salary, allowances and other conditions of service and the conditions under which they may be removed.

The functions of CEO, CAC, Scientific panels, and duties of the Authority, General Principals to be followed in the administration of the Act and a few General Provisions for Food Articles, Import, Recall and Penalties in case of non-compliance are also covered in the Act. It vests in the authority the power to make regulations, which then should be laid in front of Parliament. The State Governments are given the power to make rules and regulations under the Act.

13.2.3 THE FOOD SAFETY AND STANDARDS RULES, 2011

The Food Safety and Standards rules consist of three Chapters, 10 Forms and Memorandum of appeal. There are total 6regulations under FSSA. Each of these six sections first defines the basic terminology used in the respective section, and then states a set of regulations to be followed by the Food Business Operators (FBO), in order to be compliant to the Standards approved by the Authority and hence the Government of India. It is mandatory to be compliant to the regulations stated in the Act in order to carry out smooth, legal business in India.[9]

13.2.3.1 FOOD SAFETY AND STANDARDS (LICENSING AND REGISTRATION OF FOOD BUSINESSES) REGULATION, 2011

Every FBO is required to have a license/registration in order to carry out a food enterprise in India. This section lays down the procedures for

obtaining a license/registration. It consists of two chapters, 6 forms and 4 schedules. While the chapters give the details for obtaining license.[4] The forms include:

Form A: Application for registration/renewal of registration.
Form B: Application for registration/renewal of license.
Form C: License format.
Form D1: Annual return.
Form D2: Half-Yearly Return for Milk and Milk Products.
Form E: Form of guarantee.

Similarly various Schedules list specific details about businesses and requirements:

Schedule 1: List of food Business falling under purview of food licensing authority.
Schedule 2: Includes form A to E as given above.
Schedule 3: Fee for grant/renewal of license/registration/renewal fee.
Schedule 4: General hygiene and sanitary practices for FBOs.

13.2.3.2 FOOD SAFETY AND STANDARDS (PACKAGING AND LABELING) REGULATION, 2011

This section consists of two chapters and minute and specific details to be followed by FBOs while choosing a packaging and designing a label for the same. The requirements are product specific and depend on the type of product and the consumer targeted. It states apart from general requirements for labeling, the manner in which information is to be declared, and restrictions on specific product, lot sizes, claims, advertisements etc. to ensure no misinformation is being given to the consumers.[5]

13.2.3.3 FOOD SAFETY AND STANDARDS (FOOD PRODUCT STANDARDS AND FOOD ADDITIVES) REGULATION, 2011

Food product standards and Food Additives section consists of 3 chapters. The regulations are basically product specific safety and quality standards and also consist of an elaborative list of various food products that are being, manufactured, sold and consumed by the citizens of India. The Standards for

each of product categories and products laid down specific parameters and the range of values that those parameters should fall in with respect to those products are also given in the present section. The section has a dedicated chapter for substances added in food, which mainly regulates the type and amount of additives such as food colors, flavors, sweeteners, preservatives, and so forth added into a food product. The section also consists of two Appendixes namely A and B: Appendix A is a list of approved food additives with the food categories they are approved in; and Appendix B lists the product specific microbiological requirements that a food product should comply.

Recently, FSSAI had issued draft regulations to replace 3.1 of Chapter 3. The new regulation follows the Food Category System (FCS), which is discussed later in the chapter. The list of additives given in Appendix A has been up-graded and is a result of harmonization with Codex approved food additives. The new draft also incorporates more definitions for terms such as Maximum Use Limits (MUL), Acceptable Daily Intake (ADI), and justification for the use of additives etc. Also, it has added a table on additive category and its technological use in the product.[2]

13.2.3.4 FOOD SAFETY AND STANDARDS (PROHIBITION AND RESTRICTION ON SALES) REGULATION, 2011

Under this section, FSSAI has enlisted the prohibitions on sale of certain food articles, or certain processing conditions, or certain articles lacking some qualities that are expected to be present in their original or genuine form. Such articles either pose a threat to the health of the consumer or are of an inferior quality. Few examples of such articles are: Food resembling but not pure honey, use of carbide gas in ripening of fruits.[6]

13.2.3.5 FOOD SAFETY AND STANDARDS (CONTAMINANTS, TOXINS, AND RESIDUES) REGULATION, 2011

This section is dedicated to the terms and conditions for the usage of, or prohibition on use of certain insecticides, antibiotics and other pharmacological substances. It defines contaminants, toxins and residues and gives a detailed list of the maximum of these that are allowed in specific products.[1]

13.2.3.6 FOOD SAFETY AND STANDARDS (LABORATORY AND SAMPLING ANALYSIS) REGULATION, 2011

The laboratory and sampling analysis regulation lists down the notified, referral laboratories in different states, UTs or local areas that are accredited by FSSAI to conduct food sample testing. It states the functions of such laboratories and the procedure of sampling and testing to be followed by Food Safety officers, and the amount of samples to be sent to the laboratories.[3] The section contains two types of forms which are basically formats for the following:

Form A: Certificate of Analysis by the referral Food Laboratory
From B: Report of the Food Analyst

The Act also consists of various forms and a list of accredited laboratories that may be required for assistance of the FBOs in establishing, maintaining and carrying out their business.[3] The offences and penalties for not meeting the standards and procedures laid down by FSSA, 2006 are mentioned in Table 13.1.

TABLE 13.1 List of Offence and Penalty as per FSSA, 2006.

S. No	Clause - contravention/offence	Penalty
1	50-Food not conforming to the quality demanded	Not exceeding Rs. 25, 000/-
2	51- Sub-standard food	May extend to Rs. 5×10^5
3	52- Misbranded food	May extend to Rs. 3×10^5
4	53- Misleading advertisement	May extend to Rs. 10^6
5	54- Food containing extraneous matter	May extend to Rs. 10^5
6	55- Failure to comply with the directions of Food Safety Officer	May extend to Rs. 2×10^5
7	56- Unhygienic or unsanitary processing or manufacturing of food	May extend to Rs. 10^6
8	57- Possession of adulterant:-	(i) May extend to Rs. 2×10^5
	Adulterant not injurious to health	(ii) May extend to Rs.10^6
	Adulterant injurious to health	
9	58- Contraventions for which no specific penalty is provided	May extend to Rs. 2×10^5

TABLE 13.1 *(Continued)*

S. No	Clause - contravention/offence	Penalty
10	59- Unsafe food:- Contravention which does not result in injury Contravention resulting in a non-grievous injury Contravention resulting in a grievous injury Contravention resulting in death	i) Imprisonment up to six months and fine up to Rs.10^6 ii) Imprisonment up to 1 year and fine not exceed up to Rs. 3×10^5 iii) Imprisonment up to 6 years and fine up to Rs. 5×10^5 iv) Imprisonment not less than 7 years even or life imprisonment and fine not less Rs. 10^6.
11	60- Interfering with seized items	Imprisonment up to six months and fine up to Rs. 2×10^5
12	61- False information	Imprisonment up to three months and fine up to Rs. 2×10^5
13	62- Obstructing or impersonating Food Safety Officer	Imprisonment up to three months and fine up to Rs. 2×10^5
14	63- Carrying out a food business without license	Imprisonment up to six months and fine up to Rs. 5×10^5
15	64- Subsequent offences even after having been convicted of an offence punishable under this Act Repetition of the offence In the eventuality of continuing Repetition continuing	i) Twice punishment which might have been imposed on first conviction ii) Fine on daily basis- which may extend up to Rs. 10^6 iii) License may be cancelled
16	65- Compensation in case of injury or death:- Death of consumer Grievous injury to consumer Other case of injury to consumer	i) Not less than Rs. 5×10^5 ii) Not exceeding Rs. 3×10^5 Three Lakhs iii) Not exceeding Rs. 5×10^6
17	67- Import of article of food which is in contravention of the provisions of this Act/Rules there under	Punishable under the provisions of Foreign Trade (Development & Regulation) Act-1992 and the Custom Act-1962 as well as under this Act

Note: Indian currency Rs. 60.00 = US$1.00

13.3 HARMONIZATION WITH CODEX AND INTERNATIONAL PRACTICES

FSSR constantly strives for a set of standards that brings India at-par with the global food industry. In this context, the authority keeps conducting meetings and issuing advisories in carrying out harmonization of existing Indian Standards with Codex and other International Best Practices. A few examples of sections that have been upgraded or are under up-gradation are working methods of scientific panel, laboratory up-gradation, and additive list.[11]

13.4 INDIAN FOOD CODE (IFC)

It is a food categorization system (FCS) approach that the FSSA has implemented. Due to wide cultural and traditional diversity in food products throughout India, it is difficult to exchange data on foods, or to understand and compare the food products for their nutritional values, consumption patterns, risk analysis profiles etc. for different regions, states or individuals, without a coherent description of foods in databases. The IFC tackles issues pertaining to:

1. Clarity to all stakeholders and enforcement agencies.
2. Providing predictable, certainty and direction through cataloguing the various food products in categories in a hierarchical manner.
3. Ease in navigation throughout the catalogue by providing information in a clustered and clutter free manner.
4. Provides a direction and space for future regulatory developments.

Apart from the above, FCS will also help to describe, characterize, denominate, name, specify the product, comparison similar products, aggregate information on similar products and recovery old information. FCS will also enhance the effectiveness of official control of regulators over the entire food chain, as it is independent of creation of food product standards and covers the entire basket of products in a particular category. This means that each new product addition in market doesn't require creation of new entry in FCS.

13.5 INTERNATIONAL PRACTICES

13.5.1 NEW ZEALAND FOOD SAFETY AUTHORITY (NZFSA)

The New Zealand Food Safety Authority (NZFSA), government body of New Zealand, which is responsible for food safety and imports and exports of food and food-related products. In April 2012 it was merged into the Ministry for Primary Industries (MPI). The NZFSA has administrative legislations for food to be sold in New Zealand, primary processing of animal products and official assurances related to their export, exports of plant products and the controls surrounding registration, and use of agricultural compounds and veterinary medicines. New Food Act, 2014 introduced from March 1, 2016 will be applicable for new food businesses and suppliers who begin trading from that date. The main feature of the Food Act, 2014 is a sliding scale where businesses that are a higher risk (meals or sell raw meat or seafood) from a food safety point of view will operate under more stringent food safety requirements and checks than lower risk food businesses (non-alcoholic beverages). For effective implementation of the act to support the regulations, MPI has developed material for developing a food control plan, templates for food retail and food service activities, guidance for food businesses operating and non-operating under national programs or food control plan and for new business operators.

13.5.2 AUSTRALIAN QUARANTINE AND INSPECTION SERVICE

The Australian government agency under Department of Agriculture for enforcing quarantine laws in Australia is the Australian Quarantine and Inspection Service (AQIS). AQIS's inspection and certification is essential during import and export; and it helps Australia to maintain its highly favorable animal, plant and human health status and also an access to export markets. Risk of exotic pests and diseases is also taken care by AQIS which ultimately protects Australia's native agricultural industries and environment. Jointly Food Standards Australia and New Zealand (FSANZ) and AQIS administered the Imported Food Program, which ensures that the food which is imported always meets Australia's Quarantine Standards and the Food Standards Code.

13.5.3 MINISTRY FOR AGRICULTURE, FISHERIES, AND FORESTRY DEPARTMENT OF AGRICULTURE (DAFF)

The Australian Department of Agriculture is a department of the Government of Australia charged with the responsibility to develop and implement policies and programs that ensures Australia's agricultural, fisheries, food, and forestry industries remain competitive, profitable, and sustainable. DAFF policies and programs are involved to encourage and support sustainable natural resource use and management, protect the health and safety of plant and animal industries, to provide competitiveness for industries in fast-changing international market, provide easy market access and adaptation of new technologies.

13.5.4 FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

Food Standards Australia New Zealand (FSANZ), formerly known as Australia New Zealand Food Authority (ANZFA), is the governmental body responsible for developing food standards for Australia and New Zealand. FSANZ is a bi-national Government agency which is responsible to develop and administer the Australia New Zealand Food Standards Code. FSANZ develops food standards after consulting with other government agencies and stakeholders and has listed requirements for food products along with the usage level of additives, food safety, labeling, and GM foods. Enforcement and interpretation is the responsibility of state and territory departments and food agencies within Australia and New Zealand. The recommendations made by the body are open and accountable, and based upon a rigorous scientific assessment of risk to public health and safety.

13.5.5 ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD (ACMSF): LONDON

The ACMSF is a non-statutory committee to give an expert advice to government on issues pertaining to microbiological issues and food safety. The ACMSF was set up in 1990 and is attached with Food Standards Agency which meets quarterly at its headquarters at Aviation House, Kingsway, London. ACMSF consists of independent experts drawn from a wide range

of interests in the field of microbiology and food and has produced a number of comprehensive scientific reports.

13.5.6 NORWEGIAN MINISTRY OF AGRICULTURE AND FOOD: LANDBRUKS-OG MAT DEPARTEMENTET

The Royal Norwegian Ministry of Agriculture and Food is a Norwegian ministry established on February 17, 1900, and is responsible for agriculture, forestry, and food in Norway. The ministry is comprised of various sections which involve: Political staff, communication unit, department of administrative and economic affairs, department of forest and natural resource policy, department of food policy, department of agricultural policy, and department of research, innovation, and regional policy. The department of food policy is responsible for promotion of safe food production, animal welfare and plant and animal health. The division is also responsible for monitoring the European Economic Area Agreement (EEA) in regards of safe food production / food safety, stock reserves, health of animals, pesticides, by-products from animal processing, organic products, labeling of food for its origin and welfare of animals. The food policy department also addresses the issues related to Sanitary and phyto-sanitary Measures (SPS).

13.5.7 FEDERAL AGENCY FOR AGRICULTURE AND FOOD OR BUNDESANSTALTFÜR LANDWIRTSCHAFT UND ERNΔHRUNG (BLE)

The Federal Agency for Agriculture and Food (BLE) is a central authority with implementing various tasks in the fields of agriculture, nutrition, and consumer protection. The BLE was established on 1st January 1995. The provisions of the common agricultural and fisheries policies of the European Union and the associated control requirements guide the work of BLE. The BLE is the German market-regulating agency for the Common Market Organizations (CAP), for cereals, rice, dried fodder, sugar, fruits and vegetables, processed fruits and vegetables, live trees and other plants, bulbs, roots and the like, cut flowers and ornamental foliage, seeds, flax and hemp, hops, wine, wine alcohol, beef, pork and sheep meat, milk and milk products, and fishery products. In many cases, the BLE acts as a nationwide registration office for private inspection bodies, for example in the monitoring of organic farms and sustainable production of biofuels and biopower.

13.5.8 FEDERAL OFFICE OF CONSUMER PROTECTION AND FOOD SAFETY OR BUNDESAMTFЬR VERBRAUCHERSCHUTZ UND LEBENSMITTELSICHERHEIT (BVL)

The Federal Office of Consumer Protection and Food Safety (BVL) is a division of the Federal Ministry of Food and Agriculture belonging federal authority, based in Braunschweig. It maintains an office in Berlin. The objectives of the food, agricultural, and consumer policies of the Federal Ministry of Food and Agriculture are precautionary health protection for consumers, quality assurance and food production. Agencies and institutions under the jurisdiction include the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR), the Federal Office of Consumer Protection and Food Safety (BVL), the Federal Office of Plant Varieties (Bundessortenamt), and four federal research institutes. The BVL contributes to food safety in Germany by taking various measures. It issues approvals and coordinates monitoring programs together with the federal states.

13.5.9 FEDERAL MINISTRY OF FOOD, AGRICULTURE AND CONSUMER PROTECTION

The Federal Ministry for Food and Agriculture (***Bundesministerium für Ernährung und Landwirtschaft,*** BMEL) is a cabinet-level ministry of the Federal Republic of Germany. It consists of six departments: Central Division; Nutrition, product safety, innovation; Food safety, animal health; Rural Development, Agricultural markets; Bio-based economy, sustainable agriculture and forestry and EU policy, International cooperation, and fisheries. The BMEL and its public authorities also ensure that risks associated to the food and food products are continuously assessed, and the provisions and structures are constantly adapted to the new findings. Monitoring of compliance with these provisions is the responsibility of the industry.

13.5.10 FOOD SAFETY PROMOTION BOARD: NORTHERN IRELAND

The Food Safety Promotion Board is one of the six North South Bodies and operates under the overall policy direction of the North South Ministerial Council, with clear accountability lines back to the Council and to the

Oireachtas and the Northern Ireland Assembly. Its functions are: promoting and research in food safety, effective communication of food alerts, monitoring of foodborne disease, promotion of scientific co-operation and laboratory linkages and Development of cost-effective facilities for specialized laboratory testing.

13.5.11 EUROPEAN FOOD SAFETY AUTHORITY

The European Food Safety Authority (EFSA) is an agency of the European Union that provides independent scientific advice and communication on existing and emerging risks associated with the food chain, created by European Regulation 178/2002. EFSA was established in February 2002 and is based in Parma, Italy. EFSA's work covers all matters with a direct or indirect impact on food and feed safety and it supports the European Commission, the European Parliament and EU member states in taking effective and timely risk management decisions. EFSA also communicates to the public in an open and transparent way on all matters within its remit.[10]

13.5.12 COMMITTEE ON THE ENVIRONMENT, PUBLIC HEALTH, AND FOOD SAFETY (EU)

The Committee on the Environment, Public Health and Food Safety (ENVI) is a committee of the European Parliament having 68 members and a secretariat of 10 administrators. The committee is responsible for:

a. Environmental policy and environmental protection measures related to air, soil, and water pollution, waste management and recycling, dangerous substances and preparations, noise levels, climate change, protection of biodiversity, sustainable development, international and regional measures for protection and restoration;
b. Public health in concern with the different programs and specific actions taken, pharmaceutical and cosmetic products, health aspects of bioterrorism;
c. Food safety issues pertaining to labeling and safety of foodstuffs and veterinary legislation.

13.5.13 FOOD & DRUG ADMINISTRATION: PHILIPPINES

The Food and Drug Administration of the Philippines was created under the Department of Health. The Food and Drug Administration was established with an aim to distribute license, monitor, and regulate the flow of food, drugs, cosmetics, medical devices, and household hazardous waste in the Philippines. The FDA's main goal is to ensure the health and safety of food and drugs made available to the public.

13.5.14 THE CHINA FOOD AND DRUG ADMINISTRATION (CFDA)

The China Food and Drug Administration (CFDA), was founded on the basis of the former State Food and Drug Administration (SFDA). In March 2013, the regulatory body was rebranded and restructured as the China Food and Drug Administration, elevating it to a ministerial-level agency. The China Food and Drug Administration is directly under the State Council of the People's Republic of China, which is in-charge of comprehensive supervision on the safety management of food, health food and cosmetics and is the competent authority of drug regulation in mainland China. CFDA is responsible for drafting the laws, regulations and rules and policy plans on the administration and supervision of food safety, drugs, medical devices, and cosmetics. The other responsibilities of CFDA is to facilitate the establishment and implementation of the food safety responsibility mechanism, reporting and monitoring systems for critical food and drug; and also to take initiatives to lower down the risks associated with food and drugs.

13.5.15 GHANA FOOD AND DRUGS AUTHORITY

The Food and Drugs Authority (FDA) formerly known as Food and Drugs Board (FDB) was established in August 1997. It is the National Regulatory Authority mandated by the public Health Act, 2012 (Act 851) and is responsible to provide and enforce standards for the sale of food, herbal medicinal products, cosmetics, drugs, medical devices, and household chemical substances. The Food and Drugs Authority (FDA) as a national regulatory body that has the responsibility for the regulatory control of the manufacturing, importation, exportation, distribution, sale, and advertisement of

food, drugs, cosmetics, medical devices, and household chemical substances as enshrined in the Public Health Act, 2012 (ACT 851). Under FDA, The Food Safety Division (FSD) is established whose mandate is to protect public health and safety through the regulation. To achieve the goal of safe food FSD is comprised of Animal Products and Biosafety Department (APBD), the Food Safety Management Department (FSMD) and the Food Industrial Support Services Department (FISSD). Apart from assuring food safety FSD also provides technical support for implementing food safety management systems.

13.5.16 CANADIAN FOOD INSPECTION AGENCY (CFIA)

The Canadian Food Inspection Agency aims to mitigate risks to public health associated with diseases and other health hazards in the food supply system and to manage food safety emergencies and incidents. The CFIA achieves its objectives by promoting food safety awareness through public engagement and verification of compliance by industry with standards and science-based regulations. The Action Plan aims to strengthen Canada's food safety system by implementing stringent food safety rules, more effective inspection, a renewed commitment to service, and to disseminate information to the consumers. To achieve the goals CFIA aims to have a system capable enough to trace products in the entire production chain so that assurance is built in the system that unsafe foods are identified and removed from the supply chain as quickly as possible, and enhance industry requirements for record keeping and documentation.

13.5.17 FOOD AND DRUG ADMINISTRATION: USA

The Food and Drug Administration (FDA or USFDA) is a federal agency of the United States Department of Health and Human Services. The FDA is responsible for protecting and promoting public health through the regulation and supervision of food safety, tobacco products, dietary supplements, prescription and over-the-counter pharmaceutical drugs (medications), vaccines, biopharmaceuticals, blood transfusions, medical devices, electromagnetic radiation emitting devices (ERED), cosmetics, animal foods and feed, and veterinary products. The programs for safety regulation vary widely by the type of product, its potential risks, and the regulatory powers granted to the agency. FDA also provided with guidance and regulatory

information which are related to food safety programs, manufacturing processes, industry systems, and import/export activities.

The Center for Food Safety and Applied Nutrition (CFSAN) is the branch of FDA that regulates food, dietary supplements, and cosmetics. CFSAN is also responsible for food labeling, specifically the "Nutrition Facts" panel and deceleration of ingredients typically seen on packaged foods.

KEYWORDS

- **act**
- **food categorization system**
- **harmonization**
- **inspection**
- **licensing**
- **microbiological safety**
- **packaging and labeling**
- **regulation**

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CHAPTER 14

ENTREPRENEURSHIP AND MANAGEMENT OF FOOD PROCESSING PLANTS

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ABSTRACT

India is in reach production of raw material of food sector. There is need to utilize the raw material sources with full efficiency to avoid its loss. The best way to utilize the raw material within stipulated time period is to use automation techniques. The food processing industries are in an era of transition due to competition and a rapidly changing environment. Compared to other manufacturing industries, the food processing industries have been slow in adapting the new technologies. Computer based process control is now universally regarded as essential tool. It has forced the food industries to consider the automation of most manufacturing processes.

Automatic control tools on-line and off-line automation-computer vision system, expert systems, computer integrated manufacturing, flexible manufacturing system, robot technology, and so forth, help to improve final product quality, increase process efficiency, and reduce waste of raw materials. The application of sophisticated automation is getting more relevant to food processing industry, because it is one area of manufacturing where the quality of even the same raw material as organic farm produce keeps on varying while the finished product is always expected to be of the uniform quality. This industry therefore needs precision monitoring and maintaining the quality from the very growing of the raw materials, their harvesting and transportation, followed by processing and distribution until the finished product reaches your dining table for consumption. This trend of the automation will continue at an even faster rate in the next coming several years and would be demanding for right resources, which are well equipped and trained manpower with the newer processes and cutting edge technologies. Accordingly, the major shortage of skills in Indian food industry lies in segments like: quality control and R&D, regulatory and legal experts, production managers and engineers, shop floor technicians, marketing/sales professionals and supply chain, and logistics in-charge.

The multiplier effect of investment in food processing industry on employment generation is also higher than any other sector and hence countries like India should tap the opportunity.

14.1 INTRODUCTION

Global processed food industry has a value of US$3.2 trillion but only 6% is traded worldwide as compared to bulk agricultural commodities (16%). Across the globe, USA is largest consumer of processed food (31%) due to:

High quality, value-addition processed food is preferred over staple food, which is prevalent in less developed economies. Due to its huge potential for growth and high socio-economic impact, processed food industry is a sunrise sector. In countries where processed food industry is prevalent apart from income generation, it also helps in reduction of the postharvest losses, and increases the value of the commodity thereby fetching more foreign exchange earnings and also enhancing manufacturing competitiveness. In today's global market, quality and food safety have become competitive edge for the enterprises producing foods and providing services. With proper investment, technical innovation in food processing and infrastructure for agriculture sector, India can become the food basket of the world.[4] Therefore, food processing sector is looked upon as a job creator for people involved into agriculture for their livelihood. This is due to their familiarity with the agricultural sector, which would make it easier to train and place them in food processing enterprises.[7]

14.2 INDIAN FOOD SECTOR

India is second largest producer of food next to China. By the end of 2015, its food processing sector is expected to grow by 20% and value addition by 35%. India is the world's largest producer of milk and the second largest producer of fruits and vegetables after China. Indian Food Processing Industry had an estimated value of US$121 billion in 2012 and is expected to reach US$194 billion by 2015. The growth in food processing sector has opened up new endeavors for investors in processing, machinery, technologies, skills, and infrastructure especially in segments, which include canning, dairy, frozen food/refrigeration, and thermos-processing. India's food processing industry accounts for 32% of the total Indian food market. The sector has registered a Compound Annual Growth Rate (CAGR) of 15.6% during 2013. The industry contributes to 1.3–1.5% of India's GDP. It is ranked 5th in terms of production, consumption and exports and is dominated by Ready-to-Eat Segment (RTE), which contributes to 90% of total sales of packaged food. The food export stood at US$24.04 billion during 2012–13 and during 2008–2012, it registered a CAGR of 27.3%. During the last 5 years, the investments in this sector grew to about 20% and at present the total investment is about US$24.04 billion. In case of job opportunities, ~10 million people are employed in this sector. The Government of India (GOI) expects investments in food processing infrastructure of about US$21.9 billion by 2015. According to Department of Industrial Policy and

Promotion (DIPP), FDI worth of US$1,970.09 million have already been invested from April 2000 to July 2013.[6]

14.2.1 GROWTH OF AGRICULTURE IN INDIA

Agriculture is an important part of India's economy and at present it is among the top two farm producers in the world. Agriculture in India contributes to just over 20% of the country's GDP, but provides employment to over 50% of the population. Yield in India has gone up from 208 million tons in 2005–2006 to an estimated 263 million tons in 2013–2014. Agriculture is the only means of living for almost two-thirds of the employed class in India.[5]

14.2.2 GROWTH OF FOOD PROCESSING INDUSTRY

With the largest livestock population (485 million), India has made it largest producer of milk also (105 million tons per annum). It is second largest producer of fruits and vegetables, which accounts for 150 million tons per annum. India's export of processed food was Rs. 315.52×10^9 in 2013–2014, in which products like guar-gum gave profit worth Rs. 117.3451×10^9. India's export of dairy products was Rs. $33{,}185.3 \times 10^6$. Grain milling has the highest contribution (34%), whereas share of alcoholic beverage is lowest (3%). Sectors like fruits and vegetables, which are one of the important fields for sustainable development, contribute to only about 4%. Indian dairy industry is worth Rs. 33.1853×10^9 and it hubs more than 12 million local cooperative societies, which have created entrepreneurs with a stable source of income. Other factors, which contribute to the growth of dairy industry, are favorable demographics, fast expanding retail network, growth of user industries, and rise in bovine population.

14.2.3 DRIVERS OF FOOD PROCESSING INDUSTRIES

The food industry in India is driven by several factors like:

- Increase in spending power of the families and individuals on food products.
- Increase in number of nuclear families, entrepreneurial setups, and working women.

- Westernization and change in lifestyle.
- Increase in demand of functional foods, fresh or processed foods that claim to be health beneficial.
- Organized retail and private label penetration, which significantly affects the sale of packaged food product.
- Increasing consciousness of consumers toward healthy and special foods.
- Increased demand of packaged food in segments like low in sugar, salt, oil, preservatives free, and so forth.

14.2.4 BARRIERS AND CHALLENGES TO GROWTH OF FOOD INDUSTRIES

Despite the promising growth in food processing sector, global food wastage ranges from 15 to 45% covering all the sectors of food at all levels that is from farm to fork. The challenges for the food processing sectors are diverse and demanding, and need to be addressed on several fronts to derive maximum market benefits. A combination of uncontrollable (land holdings, climatic variations, and the constraints in land availability due to competing pressure from urbanization, constructions, and industrialization) and controllable factors (quality and quantity of raw produce, low labor productivity, infrastructure, access to credit, and inconsistency in state and central polices) has affected the growth of the sector and has acted as a hindrance in achieving its potential. Growth of food processing sector is possible on a larger scale if it is successful to overcome the following barriers and challenges:

- Share of organized retail has to be increased, for example, it is less than 5% of the total retail market in India, but is growing at over 20%.
- With dramatic shift in retail market, the demand for processed food with private retail labels is likely to increase. This would help countries like India to overcome postharvest losses of about 20–25% in fruit and vegetable segments (INR $52{,}000 \times 10^7$).
- In India nearly 90% of the food processing units are small scale and are operated by entrepreneurs with limited use of technology. The small scale entrepreneurs are able to increase the shelf-life of the food product by merely processing it for few days but it is also lost due to the poor food chain and lack of infrastructure.
- Entrepreneurs engaged in food processing usually shut down their units mainly due to cost structure as it is more vulnerable to inflation

and changing commodity prices which reduces the chances of making their venture profitable.

- The major challenges faced by entrepreneurs in food processing sector are socio-economic environment, access to credit, subsistence agriculture, fragmented value chains, lack of infrastructure for post harvest management and processing, role of federal and state government, marketing of agriculture produce, food safety regulations, availability of processing plants with cost effective technologies, cost effective food machinery and packaging technologies, and lack of skilled manpower which is required at every level.
- Affordability and price differentiation between fresh and processed food is very high relative to convenience, hygiene, and health values.
- Lack of specialized training and educational institute for catering the skill set requirements of diverse segments of food processing sector.

14.2.5 FOOD WASTAGE

The total quantity of food, which is wasted worldwide, includes: 56% wastage due to harvesting, threshing, grinding, sieving, storage, and transportation and 46% during its distribution and consumption. Inadequate storage facilities remain topic of grave concern. The Food Corporation of India (FCI) is facing two problems: (a) The production of food grains is not increasing according to the population or in other words the demand is more than its supply; and (2) FCI is not having sufficient capacity to store produced food grains. The Associated Chambers of Commerce and Industry (ASSOCHAM) predicted that combined annual production of fruits and vegetables in India is currently estimated at 227 million tons and will likely to cross the target of 377 million tons by 2021.Unfortunately, the required numbers of cold storage houses are not present in the country and the existing units are not functioning properly. In India, fresh fruits and vegetables produce worth Rs. $13{,}300 \times 10^7$ are thrown away every year due to lack of adequate cold storage facilities and refrigerated transport.

14.3 ENTREPRENEURSHIP AND MANAGEMENT OF FOOD PLANT

Without entrepreneurship and growing number of entrepreneurs, an economy can become sluggish in growth. Entrepreneurial dynamism forms the cornerstone of a progressive society as it is a purposeful activity that attempts to

create value through recognition of business opportunity, management of risk appropriate to opportunity and through communicative and management skills to mobilize human, financial, and material resources necessary to bring a project to execution.

The entrepreneurial growth in food sector has been given a high priority in India and is emerging as a strong backbone of economic growth. A food entrepreneur tries to give form or time or place utility to agricultural commodities. An entrepreneurial activity leads to introduction of useful products in the market and thereby satisfying human needs. India is rich in natural habitats and a large variety of raw material base is available which can be processed or value added upon to meet the ends. GOI is supporting the food entrepreneurs in various ways. The entrepreneurial movement began in India, in 1960s, with the establishment of *National Institute of Small Industry Extension Training* (NISIET) for promoting and encouraging entrepreneurship amongst Indian people. Few schemes like Rural Employment Generation Program (REGP) and Prime Minister's Rozgar Yojana (PMRY), act as great supportive measures for budding entrepreneurs. Industry associations such as the Confederation of Indian Industry (CII), the Federation of Indian Chambers of Commerce and Industry (FICCI) and the ASSOCHAM, and Ministry of Micro, Small & Medium Enterprises (MSME) have been working since decades in this direction.[1, 2] There are few challenges, which entrepreneurs have to face in Indian situations: The source and amount of funding; required technical know-how; ambiguous policies; insufficient R&D and research facilities; low penetration at rural level of the policies and knowledge; poor infrastructure support specially at rural areas; tough approval and funding procedures; lack of back-end and front-end linkages; lack of skilled manpower; poor technological adoption. To cover up these issues GOI has established National Innovation Council and various such bodies. Ministry of Science and Technology is taking up lots of initiatives from time to time to boost food entrepreneurship like India Innovation Growth Program, National Innovation Foundation, Technology Business Incubators operated by the Department of Science and Technology, Technology Business Incubation (TBI) program, and so forth.

There are various academic bodies in India, which train the youth to enter in this direction like National Institute of Food Technology and Entrepreneurship Management (NIFTEM), Indian Institute of Crop Processing and Technology (IICPT), and Departments of Central and State Universities. Further, it has been realized that not every person is fit to become an entrepreneur. There are some basic entrepreneurial skills needed, which if overlooked upon can lead to failures in ventures.

A food entrepreneur must have a related and appropriate outlook toward the venture. Apart from having some basic knowledge about food and food business, he/she must have a correct personality, perspective and approach toward entrepreneurship. The major traits for the entrepreneurs are broadly being covered under following six major theories:[8]

a. Economic entrepreneurship theory,
b. Psychological entrepreneurship theory,
c. Sociological entrepreneurship theory,
d. Anthropological entrepreneurship theory
e. Opportunity-based entrepreneurship theory, and
f. Resource-based entrepreneurship theory.[8]

Initially entrepreneurship was used to be considered as a substitute for academic misfit and low academic intellect, but with advent of time, entrepreneurship is emerging as a challenging and demanding domain. Any student within the fields of food technology, biotechnology, biology, business, agriculture, food sciences or related prefers to opt to be an entrepreneur and explore his/her innovative aptitude. Entrepreneurs bring innovative products in the marketplace thereby giving different directions to the Industry. There are few requirements that an entrepreneur need to understand before entering into this field: There must be a clear and strong reason to become a food entrepreneur; all necessary regulations and policies should be well understood and complied for; niche customers should be contacted regularly to have an accurate feedback; business plan should be properly laid down and worked over; Product and service knowledge should be thorough; the product should be test marketed before having plans for expansion or commercialization; selection of suppliers and service providers should be looked into carefully; strategies for marketing the product should be well decided in advance; business registration process and necessary approvals should be known; business intricacies like insurance, product licensing and labels and pricing, and so forth should be carefully decided over. Becoming a food entrepreneur is a special challenge, and to succeed preparations for the hard work and dedication are required.

The thrust for food entrepreneurship has already been realized, there is only the need to move ahead in a more systematic and structured manner. India's new food security law, along with proper training in the use of technology will prove to be a game changer in transforming the prospects of the nation as a whole. Food processing has an important role to play in linking Indian agriculture to consumers in the domestic and international markets.

However, its potential has not been tapped due to under development of the food processing sector. Entrepreneurs engaged in processed food sector can improve the status of industry by the use of latest technology in business, usage of warehouse and cold storage, reducing food wastage, and by using improved and advanced transportation and supply chain. Apart from the above-mentioned factors there is growing realization about the emerging skill shortages due to the mismatch between demand for specific skills and the available supplying food processing sector by the entrepreneurs. The acute skill deficits in the food processing sector potentially obstruct the anticipated growth rate, besides eroding industry competitiveness. The workforce lacks technical skills, knowledge and the ability to design a system, a component and a process to meet the desired needs of the industries across the food value chain (Fig. 14.1).

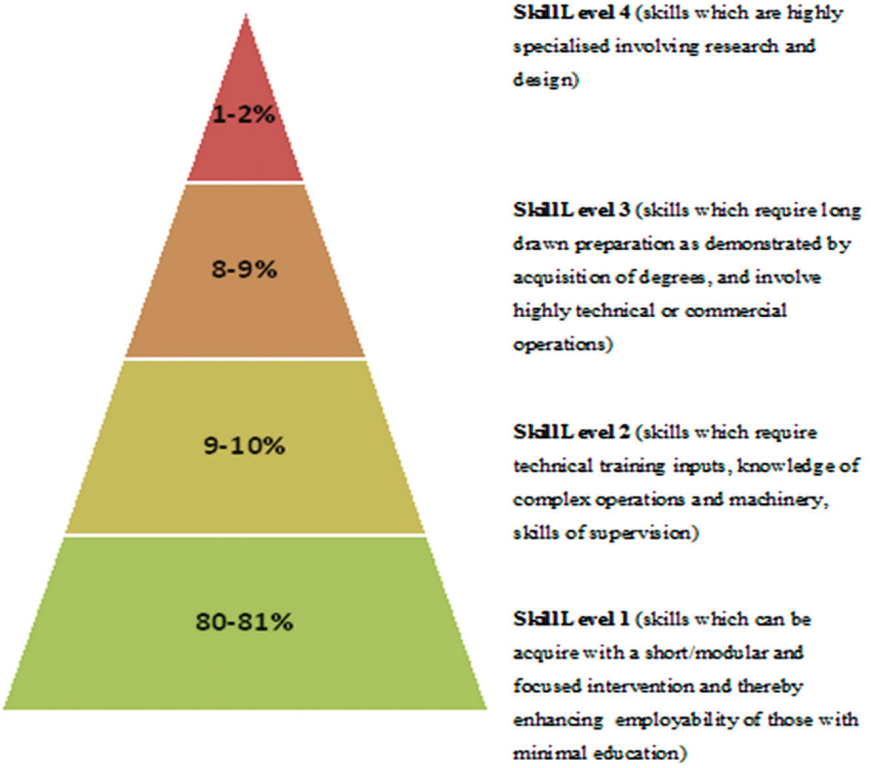

FIGURE 14.1 Skill pyramid for food processing industry.

There would be a huge spurt in the requirement of skilled manpower both at the higher end of the managerial cadre as well as in the lower end at the shop floor level. These have resulted in skill shortages due to mismatch between the demand and supply for specific skilled human resources. Shortage of skilled, semi-skilled, and unskilled workers has emerged as a critical factor which is hampering the Indian food industry. It will be a great challenge to meet the rising skill requirement and the need for trained manpower in the food processing industry.

Food processing significantly increases direct employment but it also helps to generate employment/entrepreneurship along the whole supply chain that is, raw material producers and suppliers, warehousing, distributors, transporters, and retailers. For example, a grant of INR 66.7 million invested in 35 units in Uttar Pradesh in 2005–2006 has resulted in direct employment of 2500 and indirect employment of 20,000 with a significant rural component.

14.3.1 SKILL REQUIREMENTS FOR EFFECTIVE FOOD PLANT MANAGEMENT

With advancement in technology and automation, modern food processing plants for their smooth functioning/operations demand for a technically skilled manpower. To achieve projected growth targets set, and become globally competitive, the need to develop and train the human resource would be essential for an entrepreneur engaged in processing of food. Globally it has been experienced by the entrepreneurs and the employers that skill shortages arise:

a. When they are not able to deploy staff with the appropriate skills, experience, or qualifications,
b. When their exist a skill gap or lack of skill in employees selected for specific job role, and
c. When employers are unable to recruit the required staff due to other factors like low remuneration, unsatisfactory working hours, distant location, and dearth of sector-specific specialized skills.

These gaps if not addressed well in time, the entrepreneurs engaged in food processing industry will not be able to survive for long in the global/local market. Considering the importance of the role of each employee at various stages of food processing, engineers, scientists, and technicians are becoming increasingly important due to automation and food safety

processes. These human resources include food technologist and engineers, who plan equipment layout and workflow in manufacturing plants, emphasizing efficiency and safety and also accompanied by mechanical engineers who look after the installation and maintenance of tools, equipment, and machines. Manufacturing and research and development activity at the laboratories or in the production line is the major responsibility of food scientists and technologists. Food quality assurance and managing its safety is also managed by the food technologist by minimizing the occurrence of foodborne pathogens throughout the food chain. Finally at the end of the supply chain a huge number of sales persons including wholesalers and retailers are require to push and sell the food products in the market.

Entrepreneurs now-a-days go for mechanization of food plants to overcome the low productivity and also to assure the quality of the food product. But mechanization also comes at a cost and it helps to reduce the manual work which can be replaced by computers and automation can hence result in lower wastage, lower rework and hence higher productivity. There is huge demand for skilled professionals in Indian food industry, both at the higher end technical skills and lower end skills. Food Technologists for production are likely to increase to 1,305,304 employees by 2015. With changing food safety scenario by 2015 around 1,029,182 quality assurances and R&D specialists would be required. A huge demand of supply chain and logistics professionals of ~903,672 professionals by 2015 would be required. Although, the number of experts in regulatory and legal area is relatively few, yet it is expected to reach 175,714 by 2015.[3]

14.3.2 SKILL SETS TO BE ADDRESSED IN FOOD PLANT

For food entrepreneurial setups, their success would be depending upon the skill levels of their employees and also the time in which the skill gap is reduced. For betterment of the job, the employees must always be trained as per the needs of the industry and not in a theoretical framework. Skills which should be provided to the employees can be acquired with a short/modular and focused, intervention, or through knowledge of complex operations and machinery, or by acquisition of degrees, and involve highly technical or commercial operations and by highly specialized research. Whilst the for effective implementation of skill up-gradation the managerial personal and the top management must also be well aware of the facts like food safety, hazards, auditing, inspection, product and process development, food regulation (both national and international), and export and import rules.

Some of the areas of skill building which are segment specific are given in Table 14.1, which may help the entrepreneur/employer to meet out his skill requirements.

TABLE 14.1 Skill Requirements in Different Segments of Food Processing Industry.

Segment in food processing industry	Areas for building of skills
Food grain milling industry	• Operation of power machine used for milling (knowledge of speed of operation, feeding of input, collecting output)
	• Packing: Packing of gunny bag (stitching, labeling)
Bakery-related	• Roasting/swelling to make breakfast foods
	• Mixing: Preparing flour and dough making for bread, biscuits, cakes, and so forth.
	• Making of papads, masala, and so forth.
	• Packaging and labeling
Dairy products	• Handling of milk after mulch
	• Cold storage and transportation
	• Manufacture of ice-creams and sweets
Meat and poultry processing	• Handling and slaughtering
	• Safe disposal of waste
	• Usage of by-products
Fish processing	• Preservation techniques – drying/freezing/radiation
	• Manufacturing of fish meal
	• Processing in semi-cooked and RTE forms
Fruit and vegetables and edible oil	• Drying, processing and preservation
	• Mixing in right proportions
	• Value addition that is, preparation of concentrates, juices, squash, and so forth.
	• Edible oil manufacture
Beverages (wine sector)	• Oenological practices/fermentation technology
	• HMP in whole value chain, international standards overview
	• Wine testing/sensorial analysis, handling and storage, marketing and promotion
Spices and condiments/ traditional and wellness foods	• Processing and preservation
	• Drying and aroma preservation technologies
	• Marketing and brand building
	• Value addition and value chain management

In addition to the above skills, generic skills should also be the focus for skill building in packaging (in Cans, Poly-packs), labeling, GHP, operation of food processing equipment, *Good Manufacturing Practices* (GMP), compliance to quality and safety, basic maintenance of equipment, and soft skills.

KEYWORDS

- **automation**
- **entrepreneurship**
- **food plant management**
- **food processing industry**
- **food wastage**
- **good manufacturing practices**
- **India**
- **industrialization**
- **process efficiency**
- **quality control**
- **R&D**
- **skill pyramid**
- **skill requirements**
- **skill sets**
- **supply chain**

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APPENDIX A

STANDARDS FOR DAIRY-BASED FOOD PRODUCTS

A. Dairy Products and Analogues (Regulation 5.1.1: Milk)

Boiled milk: Milk which has been brought to boil.

Double-toned milk: The product prepared by admixture of cow or buffalo milk or both with fresh skimmed milk or by admixture of cow or buffalo milk or both.

Flavored milk: The milk that may contain nuts, chocolate, coffee, or any other edible flavor, edible food colors, and cane sugar. Flavored milk shall be pasteurized, sterilized, or boiled.

Fresh skimmed milk: The product prepared by admixture of cow or buffalo milk or both that has been standardized to fat and solids-not-fat percent by adjustment of milk solids. When fat or dry non-fat milk solids are used, it shall be ensured that the product remains homogeneous and no deposition of solids takes place on standing.

Full cream milk: Milk or a combination of buffalo or cow milk or a product prepared by combination of both that has been standardized to fat and solids-not-fat percentage, by adjustment/addition of milk solids. Full cream milk shall be pasteurized. It shall be packed in clean, sound, and sanitary containers properly sealed so as to prevent contamination.

Milk: The normal mammary secretion derived from complete milking of healthy milch animal, free from colostrums, without addition thereto or extraction therefrom. Milk of different classes and designations shall conform to the standards laid down. Total urea content in the milk shall not be more than 700 ppm.

Milk products: The products obtained from milk such as cream, malai, curd, skimmed milk curd, chhanna, skimmed-milk chhanna, cheese, processed cheese, ice cream, milk ices, condensed milk sweetened and unsweetened, condensed skimmed milk-sweetened and unsweetened, milk powder, skimmed milk powder, partly skimmed milk powder, khoa, infant

milk food, table butter, and desi butter. Milk products shall not contain any substance not found in milk unless specified in the standards.

Mixed milk: A combination of milk of cow, buffalo, sheep, goat, or any other milch animal and may be a combination of any of these milk which has been made and conforms to the standards given regulations.

Pasteurization: The process of heating every particle of milk of different classes to at least 63 ˚C and holding at such temperature continuously for at least 30 minutes or heating it to at least 71.5 ˚C and holding at such temperature continuously for at least 15 seconds, or an approved temperature–time combination that will serve to give a negative Phosphatase Test. All pasteurized milk of different classes shall be cooled immediately to a temperature of 10 ˚C or less.

Recombined milk: The homogenized product prepared from milk fat, non-fat-milk solids, and water. Recombined milk shall be pasteurized and shall show a negative Phosphatase Test.

Skimmed milk: Milk from which almost all the milk fat has been removed mechanically.

Standardized milk: Cow milk or buffalo milk or sheep milk or goat milk or a combination of any of these milk that has been standardized to fat and solids-not-fat percentage given in Article 2 by the adjustment of milk solids. Standardized milk shall be pasteurized and shall show a negative Phosphatase Test.

Sterilization: Heating milk in sealed container continuously to a temperature of either 115 ˚C for 15 minutes or at least 130 ˚C for a period of one second or more in a continuous flow and then packed under aseptic condition in hermetically sealed containers to ensure preservation at room temperature for a period not less than 15 days from the date of manufacture.

Toned milk: The product prepared by admixture of cow or buffalo milk or both with fresh skimmed milk or by admixture of cow or buffalo milk or both that has been standardized to fat and solids-not-fat percentage given in Article 2 by adjustment of milk solids. It shall be pasteurized and shall show a negative Phosphatase Test. When fat or dry, non-fat-milk solids are used, it shall be ensured that the product remains homogeneous and no deposition of solids takes place on standing.

B. Cream (Regulation 5.1.2)

Cream: Cream including sterilized cream means the product of cow or buffalo milk or a combination. It shall be free from starch and other

ingredients foreign to milk. It may be of following three categories, namely: (1) Low fat cream—containing milk fat not less than 25.0% by weight; (2) Medium fat cream—containing milk fat not less than 40.0% by weight; and (3) High fat cream—containing milk fat not less than 60.0% by weight.

Cream powder: The product obtained by partial removal of water from cream obtained from milk of cow and/or buffalo. The fat and/or protein content of the cream may be adjusted by addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted. It shall be of uniform color and shall have pleasant taste and flavor free from off-flavor and rancidity. It shall also be free from vegetable oil/fat, mineral oil, added flavor, and any substance foreign to milk. The product may contain food additives permitted in these regulations. It shall conform to the microbiological requirements prescribed. It shall conform to the following requirements: (i) Moisture not more than 5.0%; (ii) Milk fat not less than 42.0%; and (iii) Milk protein in milk solid not fat not less than 34.0%.

C. Malai (Regulation 5.1.3)

Malai: The product rich in butterfat prepared by boiling and cooling cow or buffalo milk or a combination thereof. It shall contain not less than 25.0% milk fat.

D. Dahi or Curd (Regulation 5.1.4)

Dahi or curd: The product obtained from pasteurized or boiled milk by souring, natural or otherwise, by a harmless lactic acid or other bacterial culture. Dahi may contain added cane sugar. Dahi shall have the same minimum percentage age of milk fat and milk solids-not-fat as the milk from which it is prepared. Where dahi or curd is sold or offered for sale without any indication of class of milk, the standards prescribed for dahi prepared from buffalo milk shall apply. Milk solids may also be used in preparation of this product.

E. Chhana or Paneer (Regulation 5.1.5)

Chhana or paneer: The product obtained from the cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid, or citric

acid. It shall not contain more than 70.0% moisture and the milk fat content shall not be less than 50.0% of the dry matter. Milk solids may also be used in preparation of this product. Provided that paneer or chhana when sold as low fat paneer or chhana, it shall conform to the following requirements:(i) Moisture not more than 70.0% and (ii) Milk fat not more than 15.0% of dry matter.

F. Cheese (Regulation 5.1.6)

Cheese 1 (hard): The product obtained by draining after coagulation of milk with a harmless milk-coagulating agent under the influence of harmless bacterial culture. It shall not contain ingredients not found in milk, except coagulating agent, sodium chloride, and calcium chloride not exceeding 0.02% by weight, annatto or carotene color, and may contain emulsifiers and/or stabilizers, namely citric acid, sodium citrate, or sodium salts of orthophosphoric acid and polyphosphoric acid (as linear phosphate) exceeding 0.2% by weight. Wax used for covering the outer surface shall not contain any thing harmful to health. In case the wax is colored, only permitted food color shall be used. Hard cheese shall contain not more than 43.0% moisture and not less than 42.0% milk fat of the dry matter. Hard cheese may contain up to 3000 parts per million sorbic acid, or its sodium, potassium or calcium salts calculated as sorbic acid, and/or 12.5 parts per million nisin either singly or in combination. Natamycin may be used for surface treatment only, subject to the following conditions, namely: (i) Maximum level of application shall not exceed 2 mg/dm^3 of cheese surface, (ii) The penetration depth shall not exceed 2 mm, and (iii) The maximum residue level in the finished product shall not exceed 1 mg/dm^3.

Cheese 2: The ripened or unripened soft or semi-hard, hard, or extra hard product, which may be coated with food grade waxes or polyfilm, and in which the whey protein/casein ratio does not exceed that of milk. Cheese is obtained by coagulating wholly or partly milk and/or products obtained from milk through the action of non-animal rennet or other suitable coagulating agents and by partially draining the whey resulting from such coagulation and/or processing techniques involving coagulation of milk and/or products obtained from milk which give a final product with similar physical, chemical, and organoleptic characteristics. The product may contain starter cultures of harmless lactic acid and/or flavor producing bacteria and cultures of other harmless microorganisms, safe and suitable enzymes and sodium chloride. It may be in the form of blocks, slices, cut, shredded, or grated cheese.

1. **Mould ripened** cheese is a ripened cheese in which the ripening has been accomplished primarily by the development of characteristic mould growth through the interior and/or on the surface of the cheese.
2. **Ripened cheese** is cheese which is not ready for consumption shortly after manufacture but which must be held for some time at such temperature and under such other conditions as will result in necessary biochemical and physical changes characterizing the cheese in question.
3. **Unripened cheese** including fresh cheese is cheese which is ready for consumption shortly after manufacture.

Cheese or varieties of cheeses shall have pleasant taste and flavor free from off-flavor and rancidity. It may contain food permitted additives**.** It shall conform to the prescribed microbiological requirements.

Brie cheese means soft ripened cheese obtained by coagulating milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria and cultures of Penicillium caseicolum and Bacterium linens, non-animal rennet, and other suitable enzymes. It shall be white to creamy yellow in color with a smooth texture showing presence of white mould (Penicillium caseicolum) with occasional orange colored spots (Bacterium linens) on the rind. It shall conform to the following requirements: (i) Moisture not more than 56.0%; (ii) Milk fat on dry basis not less than 40.0%.

Camembert cheese means ripened soft cheese obtained by coagulating milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria and cultures of Penicillium caseicolum and Bacterium linens non-animal rennet, or other suitable coagulating enzymes. It may be in the form of flat cylindrical shaped cheese covered with white mould (Penicillium caseicolum) with occasional orange colored spots (Bacterium linens).

Cheddar cheese: Ripened hard cheese obtained by coagulating heated/pasteurized milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria, non-animal rennet, or other suitable coagulating enzymes. It shall be in the form of hard pressed block with a coating of food grade waxes or wrapping of cloth or polyfilm. It shall have firm, smooth and waxy texture with a pale straw to orange color without any gas holes. It shall conform to the following requirements: (i) Moisture—not more than 39.0%; (ii) Milk fat on dry basis—not less than 48.0%.

Cottage cheese and creamed cottage cheese means soft unripened cheese obtained by coagulation of pasteurized skimmed milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid bacteria with or without the addition of other suitable coagulating enzymes. Creamed cottage cheese is cottage cheese to which a pasteurized creaming mixture of cream, skimmed milk, condensed milk, non-fat dry milk, dry milk protein, and sodium/potassium/calcium/ammonium caseinate is added. It shall have a soft texture with a natural white color. It may contain spices, condiments, seasonings, and fruits pulp. It may contain food additives permitted in these regulations. It shall conform to the following requirements: (i) Moisture not more than 80.0%; (ii) Milk fat (in creamed cottage cheese) not less than 4.0%.

Coulommiers cheese means soft unripened cheese obtained by coagulation of milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria and non-animal rennet, or other suitable coagulating enzymes and moulds characteristic of the variety. It shall have soft texture and white to cream yellow color and may show presence of white mould including orange or red spots on the surface. It may contain food additives permitted in these regulations. It shall conform to the following requirements: (i) Moisture not more than 56.0%; (ii) Milk fat on dry basis not less than 46.0%.

Cream cheese (Rahmfrischkase) means soft unripened cheese obtained by coagulation of pasteurized milk of cow and/or buffalo or mixtures thereof and pasteurized cream with cultures of harmless lactic acid-producing bacteria with or without the addition of suitable coagulating enzymes. It shall have a soft smooth texture with a white to light cream color. It may contain spices, condiments, seasonings, and fruits pulp. The product may contain food additives permitted in these regulations. It shall conform to the following requirements: (i) Moisture not more than 55.0% and (ii) Milk fat on dry basis not less than 70.0%.

Danbo cheese: Ripened semi-hard cheese obtained by coagulating heated/pasteurized milk of cow and/or buffalo and mixtures thereof with cultures of harmless lactic acid-producing bacteria, non-animal rennet, or other suitable coagulating enzymes. It shall be smooth in appearance with firm texture and uniform yellow color and may be coated with food grade waxes or wrapping of cloth or polyfilm. It may contain food additives permitted in these regulations.

Edam cheese means the ripened semi-hard cheese obtained by coagulating heated/pasteurized milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria non-animal rennet,

or other suitable coagulating enzymes. It shall have a firm texture suitable for cutting with a yellowish color and a hard rind which may be coated with food grade waxes, wrapping of cloth, polyfilm, or vegetable oil. It shall conform to the following requirements: (i) Moisture—not more than 46.0%; (ii) Milk fat (dry basis): not less than 40.0%.

Emmentaler: Hard ripened cheese with round holes obtained by coagulating milk of cow and/or buffalo or mixtures thereof with non-animal rennet, cultures of harmless lactic acid-producing bacteria, or other suitable coagulating enzymes. It may contain cupric sulfate not exceeding 15 mgm/kg expressed as copper. It shall have a light yellow color and a firm texture suitable for cutting and may have a hard rind. It may contain food additives permitted in these regulations.

Gouda cheese: Ripened semi-hard cheese obtained by coagulating milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria non-animal/rennet, or other suitable coagulating enzymes. It shall have firm texture suitable for cutting, straw to yellowish color, and a hard rind which may be coated with food grade waxes, wrapping of cloth, or vegetable oil.

Havarti cheese: Ripened semi-hard cheese obtained by coagulating milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria, non-animal rennet, or other suitable coagulating enzymes. It shall have firm texture suitable for cutting, a light yellow color, and may have a semi-soft slightly greasy rind. It may contain food additives permitted in these regulations.

Provolone: Pasta filata cheese obtained by coagulating milk of cow and/or buffalo or mixtures thereof with cultures of harmless lactic acid-producing bacteria, non-animal rennet, or other suitable coagulating enzymes. It may be smoked. It shall be white to yellow straw in color with a fibrous or smooth body and rind which may be covered with vegetable fat/oil, food grade waxes, or polyfilm. It may contain food additives permitted in these regulations.

G. Dairy-based Desserts/Confections (Regulation 5.1.7)

Dried ice cream mix/dried frozen dessert/confection-1: The product in a powder form which on addition of prescribed amount of water shall give a product conforming to the requirements of the respective products, namely—ice cream, medium fat ice cream, low fat ice cream as prescribed under regulation and frozen confection, medium fat frozen confection, and

low fat frozen confection as prescribed under regulation of these regulations except the requirement of weight/volume for both the products. The moisture content of the product shall not be more than 4.0%.

Dried ice cream mix-1 shall be the material prepared by spray or other roller drying of ice cream mix. It shall contain milk solids, sucrose, or corn syrup or refined sugar. It may contain permitted colors and flavors. It may contain stabilizers and emulsifiers not exceeding 1.25% by weight. The product shall contain not less than 27.0% milk fat and 9.5% protein and moisture shall not be more than 4% by weight. The sucrose content shall be not more than 40% by weight. It shall be packed in hermetically sealed containers.

Frozen dessert/frozen confection-3: The product obtained by freezing a pasteurized mix prepared with milk fat and/or edible vegetable oils and fat having a melting point of not more than 37 ˚C in combination and milk protein alone or in combination/or vegetable protein products singly or in combination with the addition of nutritive sweetening agents. It may also contain chocolate, cake, or cookies as a separate layer or coating. It may be frozen hard or frozen to a soft consistency. It shall be free from artificial sweetener. It shall have pleasant taste and flavor free from off-flavor and rancidity. The product may contain food additives permitted in these regulations. It shall conform to the microbiological requirements prescribed.

Ice cream, kulfi and chocolate-1 ice cream: Frozen product obtained from cow or buffalo milk or a combination or from cream and/or other milk products, with or without the addition of cane sugar, dextrose, liquid glucose, and dried liquid glucose, Maltodextrin, fruits, fruit juices, preserved fruits, nuts, chocolate, edible flavors, and permitted food colors. It may contain permitted stabilizers and emulsifiers not exceeding 0.5% by weight. The mixture shall be suitably heated before freezing. The standards for ice cream shall also apply to softy ice cream. In case of ice cream, where the chocolate or like covering portion forms a separate layer, only the ice cream portion shall conform to the standards of ice cream.

Ice cream, kulfi, chocolate ice cream, or softy ice cream-2: The product obtained by freezing a pasteurized mix prepared from milk and/or other products derived from milk with addition of nutritive sweetening agents e.g. sugar, dextrose, fructose, liquid glucose, liquid glucose, high-maltose corn syrup, honey, fruit and fruit products, eggs and egg products, coffee, cocoa, ginger, and nuts. It may also contain chocolate, and bakery products such as cake, or cookies as a separate layer and/or coating.

Khoya (pindi, danedar, dhap, mawa, kava): The product obtained from cow or buffalo or goat or sheep milk or milk solids or a combination thereof

by rapid drying. The milk fat content shall not be less than 30% on dry weight basis of finished product. It may contain citric acid not more than 0.1% by weight. It shall be free from added starch, added sugar and added coloring matter.

H. Evaporated/Condensed Milk and Milk Products (Regulation 5.1.8)

Evaporated milk: The product obtained by partial removal of water from milk of cow and/or buffalo by heat or any other process which leads to a product of the same composition and characteristics. The fat and protein content of the milk may be adjusted by addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted. It shall have pleasant taste and flavor free from off-flavor and rancidity.

Milk powder: The product obtained by partial removal of water from milk of cow and/or buffalo. The fat and/or protein content of the milk may be adjusted by addition and/or withdrawal of milk constituents in such a way not to alter the whey protein to casein ratio of the milk being adjusted. It shall be of uniform color and shall have pleasant taste and flavor free from off-flavor and rancidity. It shall be free from vegetable oil/fat, mineral oil, thickening agents, added flavor, and sweetening agent. It may contain food additives permitted in regulations.

Sweetened condensed milk: A product obtained by partial removal of water from milk of cow and/or buffalo with the addition of sugar or a combination of sucrose with other sugars or by any other process which leads to a product of the same composition and characteristics. The fat and/or protein content of the milk may be adjusted by addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted. It shall have pleasant taste and flavor free from off-flavor and rancidity. It shall be free from any substance foreign to milk. It may contain food additives in permitted regulations.

REFERENCE

Food Safety and Standards Regulations (2010). *The Gazette of India Extraordinary [Part III—Sec. 4],* pp 410–776. Ministry of Health and Family Welfare, New Delhi, India, 20 October, 2010.

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APPENDIX B

SPECIFIC HYGIENIC AND SANITARY PRACTICES TO BE FOLLOWED BY FOOD BUSINESS OPERATORS ENGAGED IN MANUFACTURE, PROCESSING, STORING, AND SELLING OF MILK AND MILK PRODUCTS

In addition to following all other regulatory requirement as given by various laws, a dairy establishment in which dairy-based food is being handled, processed, manufactured, stored, distributed, and ultimately sold by the food business operator, and dairy should conform to the sanitary and hygienic requirement, food safety measures and other standard as specified in the following paragraphs:

A. Sanitary Requirements

1. Dairy establishments shall have the following:

    a. Facilities for hygienic handling and protection of raw materials and of non-packed dairy products in loading and unloading, transport, and storing bulk milk cooling facilities.
    b. Appropriate arrangements for protection against pests are must.
    c. Instruments and equipment intended to come into direct contact with raw materials and dairy products which are made of corrosion-resistant material, easy to clean and disinfect.
    d. Special watertight non-corrodible containers to put raw materials and dairy products. Where such raw materials/dairy products are

removed by conduits, shall be constructed and installed to avoid risk of contamination.

e. Appropriate facilities for the cleaning and disinfecting of equipment and instruments especially cleaning in place (CIP) system.
f. Wastewater disposal system which is hygienic and approved by Pollution Control Board.
g. Lockable room/secure place for storage of detergents, disinfectants and other things.
h. Facilities for cleaning and disinfecting of tanks used for transporting dairy products and raw milk. These containers have to be cleaned after every use.

2. Dairy establishments shall have working areas of sufficient size for work to be carried out under adequate hygienic conditions; their design and layout shall be such as to preclude contamination of the raw materials and the dairy products.
3. In areas where dairy products are manufactured, the areas shall have the following:

a. Solid, waterproof flooring which is easy to clean and disinfect and which allows water to drain away, and equipment to remove water.
b. Walls with smooth surfaces and which are easy to clean, are durable and impermeable.
c. Covered with light-colored coating.
d. Ceilings/roof linings which are easy to clean in those areas where exposed/non-packaged raw materials/dairy products are handled.
e. Doors made of non-corrodible materials which are easy to clean.
f. Ventilation and, where necessary, good steam and water-vapor extraction facilities in accordance with Factory Act, 1948.
g. Adequate natural or artificial lighting in accordance with Factory Act, 1948.
h. An adequate number of facilities with hot and cold running water, water pre-mixed to a suitable temperature, for cleaning and disinfecting hands; taps in work rooms and lavatories for cleaning and disinfecting hands which shall be non-hand-operable, these facilities shall be provided with cleaning and disinfecting materials and a hygienic.
i. Facilities for cleaning tools, equipment, and installations.

4. The occupier of a dairy establishment shall take appropriate measures to avoid cross-contamination of dairy products in accordance with the cleaning program specified earlier.
5. Where a dairy establishment produces foodstuffs containing dairy products together with other ingredients, which have not undergone heat treatment/any other treatment having equivalent effect, such dairy products, shall be stored separately to prevent cross-contamination.
6. Production of heat-treated milk/manufacture of milk-based products, which might pose a risk of contamination to other dairy products, shall be carried out in a clearly separated working area.
7. Instruments and equipment used for working on raw materials and dairy products, floors, ceilings or roof linings, walls and partitions shall be kept in a satisfactory state of cleanliness and repair, so that they do not constitute a source of contamination to raw materials or dairy products.
8. Equipment, containers, and installations which come into contact with dairy products or perishable raw materials used during production shall be cleaned and if necessary disinfected according to a verified and documented cleaning program.
9. Equipment, containers, instruments, and installations shall be cleaned and disinfected according to a verified and documented Food Safety management system program drawn up by the occupier of the dairy establishment.
10. The processing establishment shall in principle be cleaned according to an established, verified, and documented Food safety management program. The manufacturer shall take appropriate measures to avoid any kind of cross-contamination.
11. Disinfectants and similar substances used shall be used in such a way that they do not have any adverse effects on the machinery, equipment, raw materials, and dairy products kept at the dairy establishment.
12. Any container or tank used for transporting or storage of raw milk shall be cleaned and disinfected before reuse.

B. Personal Hygiene Requirements

1. The FBO shall employ those persons only in such an establishment to work directly with and handle raw materials/dairy products if those persons have proved to the occupier's satisfaction by means of a medical certificate that there is no medical impediment to their employment.

2. Persons working directly with and handling raw materials or dairy products shall maintain the highest standards of personal cleanliness at all times. In particular they shall:

    a. Wear suitable, clean working clothes and headgear which completely encloses their hair.
    b. Not smoke, spit, eat, or drink in rooms where raw materials and dairy products are handled or stored.
    c. Wash their hands at least each time work is resumed and whenever contamination of their hands has occurred; e.g. after coughing/sneezing, visiting toilet, using telephone, smoking etc.
    d. Cover wounds to the skin with a suitable waterproof dressing. No person with injury on hand, even with dressing, shall be placed in any product making/handling section.
    e. Avoid certain hand habits—e.g. scratching nose, running finger through hair, rubbing eyes, ears and mouth, scratching beard, scratching parts of bodies etc. that are potentially hazardous. When unavoidable, hands should be effectively washed before resuming work.

3. The occupier shall take all necessary measures to prevent persons liable to contaminate raw materials and dairy products from handling them until the occupier has evidence that such persons can do so without risk of contamination.

C. Sanitary Requirements for Storage

1. Immediately after procuring, raw milk shall be placed in a clean place, which is suitably equipped so as to prevent any kind of contamination.
2. Where raw milk is collected daily from a producer, it shall, if not collected and brought to the dairy plant within four hours of milking, be cooled as soon as practicable after procuring to a temperature of 4 ºC and maintained at that temperature until processed.
3. Upon acceptance at a processing establishment milk shall, unless heat-treated immediately, be cooled to a temperature of 4 ºC or lower, if not already at such temperature, and maintained at that temperature until heat-treated.
4. When the pasteurization process is completed, pasteurized milk shall be cooled immediately to a temperature of 4 ºC or lower.

5. Subject to Paragraph 7 below, any dairy product not intended to be stored at ambient temperature shall be cooled as quickly as possible to the temperature established by the manufacturer of that product as suitable to ensure its durability and thereafter stored at that temperature.
6. Where dairy products other than raw milk are stored under cooled conditions, their storage temperatures shall be registered and the cooling rate shall be such that the products reach the required temperature as quickly as possible.
7. The maximum temperature at which pasteurized milk may be stored until it leaves the treatment establishment shall not exceed 5 ºC.
8. Dairy products not intended to be stored at ambient temperature shall be cooled as quickly as possible to the temperature established by the manufacturer of that product as suitable to ensure its durability and shelf life.
9. Where dairy products other than raw milk are stored under cool conditions, their storage temperatures shall be recorded and the cooling rate shall be adjusted in such a way that the products reach the required temperature at the earliest.

D. Wrapping and Packaging

1. The wrapping packaging of dairy products shall take place under satisfactory hygienic conditions and in rooms provided for that purpose.
2. The manufacture of dairy products and packaging operations may take place in the same room if the following conditions are satisfied:

    a. The room shall be sufficiently large and equipped to ensure the hygiene of the operations;
    b. The wrapping and packaging shall have been brought to the treatment or processing establishment in protective cover in which they were placed immediately after manufacture and which protects the wrapping or packaging from any damage during transport to the dairy establishment, and they shall have been stored there under hygienic conditions in a room intended for that purpose;
    c. The rooms for storing the packaging material shall be free from vermin and from dust which could constitute an unacceptable risk of contamination of the product and shall be separated from rooms containing substances which might contaminate the products. Packaging shall not be placed directly on the floor;

d. Packaging shall be assembled under hygienic conditions before being brought into the room, except in the case of automatic assembly or packaging, provided that there is no risk of contamination of the products;
e. Packaging shall be done without delay. It shall be handled by separate group of staff having experience in handling and product wrapping, and
f. Immediately after packaging, the dairy products shall be placed in the designated rooms provided for storage under required temperature.

3. Bottling or filling of containers with heat-treated milk and milk product shall be carried out hygienically.
4. Wrapping or packaging may not be re-used for dairy products, except where the containers are of a type which may be re-used after thorough cleaning and disinfecting.
5. Sealing shall be carried out in the establishment in which the last heat-treatment of drinking milk or liquid milk-base products has been carried out, immediately after filling, by means of a sealing device which ensures that the milk is protected from any adverse effects of external origin on its characteristic.

REFERENCE

Food Safety and Standards Regulations (2010).*The Gazette of India Extraordinary: The 20 October, 2010*. Ministry of Health and Family Welfare, Food Safety and Standards Authority of India, New Delhi, India.

APPENDIX C

LIST OF DAIRY PRODUCTS

Dairy product	Country of origin
Aarts	
Aaruul	
Amasi	South Africa
Ayran	Turkey
Baked milk	Eastern Europe
Balmithai	India
Basundi	India
Bhuna khoya	Pakistan
Blaand	Scotland
Black Kashk	Central Asia
Booza	
Bulgarian yogurt	Bulgaria
Butter	
Butter oil	
Buttermilk	
Cacık	Turkey
Caudle	
Chaas	India
Chal	
Chalap	
Cheese	
Chhana	India

Dairy product	Country of origin
Chortan	
Clabber	
Clotted cream	
Condensed milk	
Cream	
Cuajada	Spain
Curd	
Curd snack	
Custard	
Dadiah	Indonesia
Daigo	Japan
Dairy spread	
Dondurma	Turkey
Doogh	
Evaporated milk	
Feta	Greece
Filled milk	
Frozen custard	
Frozen yogurt	United States
Galalith	
Gelato	Italy
Ghee	India
Gombe	Norway
Gulabjamun	India
Ice cream	
Junket	
Kashk	
Kaymak	Turkey

Dairy product	Country of origin
Kefir	Caucasus
Khoa	India
Kulfi	India
Kumis	Mongolia
Lassi	India
Malai	India
Matzoon	Armenia
Milk	
Milk powder	
Míša	Czech Republic
Moose milk	
Mursik	Kenya
Paneer	India
Peda	India
Pomazánkové máslo	
Pytia	
Qatiq	
Qimiq	
Quark	
Qurut	
Ryazhenka	Ukraine
Semifreddo	Italy
Sergem	Tibet
Shrikhand	India
Skim milk powder	
Skorup	
Skyr	Iceland
Smetana	Europe

Dairy product	Country of origin
Soft serve	United States
Stewler	Ukraine, Russia
Strained yogurt	
Súrmjólk	Iceland
Tarhana	
Tuttis	
Urdă	Romania
Uunijuusto	Finland
Viili	
Vla	Netherlands
Whey	
Whipped cream	
Yak butter	
Ymer	Denmark
Yoghurt	
Žinčica	

This list is compiled based on information from:

1. en.wikipedia.org/wiki/List_of_dairy_products
2. en.wikipedia.org/wiki/Dairy_product
3. en.m.wikipedia.org/wiki/Category:Dairy_products
4. oregonuncovered.com/…16280/list-dairy-products-wiki

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