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Milk Processing and Dairy Products Industry

Edited by Roua Lajnaf



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Aims and Scope of the Series

The significance of food is undeniable, especially in light of the impending challenge facing humanity: ensuring there will be enough food to meet the basic needs of a population expected to reach approximately 10 billion by 2050. These food-related challenges align with some of the United Nations' sustainable development goals, with a target to achieve them by 2030. One thing is certain: food should be not only nourishing and safe but also tailored to the diverse needs of individuals throughout their lifetimes, all while meeting consumers' sensory expectations. Understanding the diverse chemical composition of food, often referred to as biodiversity, and how these components can contribute to human health by considering factors like bioaccessibility, bioavailability, and bioactivity at the organ level, is crucial for grasping and promoting a healthy diet. Thanks to the continuous evolution of analytical methods and interdisciplinary research, significant strides have been made in the field of food science and nutrition.

Meet the Series Editor



Maria Rosário Bronze has been working in Analytical Chemistry since 1986. Her Ph.D. in 1999 contributed to the study of food products using capillary electrophoresis. The main goal of her research since 1999 has been focused on Analytical Chemistry applied mainly to the analysis of foods and by-products of food industry. She conducted research in collaboration with national and international research groups, at iBET and ITQB Technology Division. From 2017 until 2021 she was head of Food & Health Division at iBET and head of the Food Functionality and Bioactives Laboratory. MR Bronze has been an Associate Professor at the Pharmacy Faculty of Lisbon University and head of the Structural Analysis Laboratory since 2012. As a researcher, MR Bronze is a Senior Scientific Advisor at Food & Health Division at iBET and Head of Food Functionality and Bioactives Laboratory at the same Institute, Collaborator at iMED and Researcher at ITQB NOVA. Her current research is focused on quality and beneficial health effects of food components. Gas and liquid chromatography associated with mass spectrometry are used by MR Bronze in the characterization of samples. Sensory evaluation is also an important area of her research. The main food products studied by her are olive tree products (olive, olive oil, leaves), cereals such as maize, legumes (faba bean, pea, chickpea, lentils) fruits (apple, grapes, opuntia ficus), fruit juices and wine, among others. More recently her interests have also involved biodiversity, bioaccessibility, and bioavailability studies on food products and their components, mainly phytochemicals as phenolic compounds, using different analytical tools such as mass spectrometry. As a senior scientific advisor at Food & Health Division at iBET she is involved in different areas: (i) isolation, characterization and formulation of bioactive and functional compounds or extracts from natural sources and wastes from food and other related industries; (ii) pre-clinical assays to provide support to understand health claims related with the beneficial effects of food nutrients/bioactive components; (iii) establishment of analytical methodologies including mass spectrometry state-of-the-art to fully characterize different matrices, from food products, natural extracts or biological fluids (Food Functionality and Bioactives Laboratory).

Meet the Volume Editor



Dr. Roua Lajnaf holds an Engineer and MSc degree in Biological Engineering - Food Biochemistry from the National Engineering School of Sfax, Tunisia, and a Ph.D. in Biochemistry and Food Technology from the University of Montpellier, France, obtained in 2017. Her areas of expertise span the dairy industry, milk proteins, and the impact of various food processing technologies on their techno-functional properties. Dr. Lajnaf has significant academic and industrial experience in Food Technology and Food Processing. She has taught at the Universities of Sfax, Monastir, and Tunis El-Manar in Tunisia, covering topics such as food technology, food industry operations, quality management, and nutritional pathologies, particularly food allergies. She has actively participated in numerous conferences, seminars, and congresses. She has an extensive publication record, including research articles, review papers, book chapters, books, and patents in the fields of food engineering and food allergies. Additionally, she serves as a reviewer for various high-impact journals in food processing and technologies. Dr. Lajnaf is the editor of several prominent books and the Lead Guest Editor for journals specializing in nutrition and Food sciences. Her excellence in the field was recognized with the prestigious L'Oréal-UNESCO For Women in Science Prize in 2022 for her contributions to the field of Biological Sciences. Currently, Dr. Lajnaf is leading a research project, *The Effect of Climate Change on Milk Quality and Technological Properties*, further solidifying her reputation as a key figure in food technology and dairy science.

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Preface

The book *Milk Processing and Dairy Products Industry* provides an in-depth description and the latest research on milk preservation processes and the manufacturing steps of various dairy products, from the receipt of raw materials to packaging. *Milk Processing and Dairy Products Industry* serves as an authoritative reference, bridging the gap between theoretical knowledge of dairy chemistry and its practical applications in the dairy industry.

This unique book is up-to-date and properly referenced for accessibility to all scientific and industrial readers. It covers the definition, types, descriptions, global production, manufacturing process, and physicochemical properties, as well as factors with significant impact on the physicochemical, nutritional, and microbiological quality of dairy products in the dairy industry, such as cheese, yoghurt, cream, butter, ice cream, condensed, and dried milk. Indeed, milk processing can have many beneficial effects in producing safe dairy products and stabilizing them against alteration. To the best of my knowledge, there have been few books written on milk dairy products that combine both scientific and industrial sides. Hence, the aim of both industry professionals and scientists is not only to enhance the sustainability of the dairy supply chain but also to preserve the quality attributes of dairy products through the implementation of innovative and sustainable processing technologies.

This unique book provides an authoritative source of information on milk processing techniques used in dairy product manufacturing, offering valuable insights for the research, industrial, and commercial communities. The content of *Milk Processing and Dairy Products Industry* includes the latest research on preservation techniques in the dairy industry, encompassing both thermal and non-thermal methods, as well as fermentation processes and the physicochemical and microbiological quality of dairy products derived from cow's milk and other mammalian species, such as goat milk. Thus, each chapter in this book was prepared by dairy scientists with great expertise.

Finally, the book *Milk Processing and Dairy Products Industry* is the result of collaborative efforts by numerous experts, each bringing their specialized knowledge, culminating in a high-quality scientific work. These experts are affiliated with industry, research institutions, and academia from many countries worldwide. Hence, we thank all the contributing lead authors and co-authors for sharing their

knowledge and expertise, for their invaluable contributions to the dairy products field, and for their patience in staying on course and seeing this project through to completion.

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Section 1

Advances in Dairy Processing Technologies

Introductory Chapter: Novel Processing Technologies in the Dairy Product Industry

Roua Lajnaf

1. Introduction

Milk has been processed into various dairy products using traditional methods for hundreds of years. Currently, consumers are becoming increasingly demanding about the quality of industrial dairy products. Thus, scientific progress has focused on the development of new food processing techniques in dairy that can both ensure food safety and prevent the degradation of nutrients such as proteins and vitamins, thereby enhancing the health benefits and nutritional value of manufactured dairy products [1]. Numerous technological advancements have focused on unit operations, including pasteurization, sterilization, standardization, separation, and packaging, resulting in considerable improvements in hygiene, product quality, mechanization, automation, and energy efficiency within processing technologies in the dairy industry [2]. At the same time, food engineering in the dairy industry leverages modern processes, tools, technologies, and knowledge to develop new products aimed at enhancing consumer perception and convenience, while also improving the cost-effectiveness of production [3].

Overall, the main and common objective of industrialists in the dairy industry is to effectively combine an extended shelf life of the manufactured product with the preservation of its nutritional and organoleptic properties. The use of thermal treatments such as sterilization and pasteurization has consistently been a standard practice in the dairy industry, ensuring the microbiological quality of the resulting dairy products [4]. However, scientific advancements have led to significant developments in thermal processing techniques in the dairy industry, aiming to minimize the damage that traditional thermal treatments may cause to the nutritional and organoleptic properties of the products. These novel thermal treatments include ohmic treatment and infrared heating which aim to serve as effective alternatives to traditional preservation technologies [1, 5].

Meanwhile, other technological processes in the food industry, including the dairy industry, have been developed. These technologies are characterized by the absence of heat, hence their name “non-thermal processing technologies.” They treat food products at ambient temperature with the aim of minimizing the undesirable effects that heat could cause, such as the degradation of sensory quality and the reduction of health benefits [5]. On the other hand, membrane technology is also used in the dairy industry for fractionation or separation purposes, depending on the applied pressure and the membrane pore size and. For instance, microfiltration, involving membranes with a pore size that ranges between 0.1 and 10 μm , is efficient at removing bacteria and spores from skim milk; thus, this process is called cold pasteurization [6].



Figure 1. Main benefits (blue box) and drawbacks (orange box) of novel thermal and nonthermal processing technologies in the dairy industry. Abbreviations: OHT: ohmic heating technology; IH: infrared heating, MWP: microwave processing; HPP: high-pressure processing, PEF: pulsed electric field; MF: membrane filtration.

Finally, driven by a focus on efficiency, the dairy industry has experienced consistent growth over the past 5 years, even as the economy has gradually recovered from a significant downturn. While food engineering in the dairy sector offers numerous advantages, it also presents certain many challenges. Therefore, the aim of this introductory chapter is to provide readers with a comprehensive overview of the common novel thermal and nonthermal processing technologies of the dairy industry. It aims to offer readers a balanced perspective on the advantages and limitations of these technologies, as summarized in **Figure 1**, supported by examples from recent research.

2. Novel thermal processing technologies

Thermal treatment technologies have long been the preferred method for processing foods, ensuring its safety for consumption and helping to prolong its shelf-life [7]. However, milk can undergo alteration upon thermal treatments including biological and chemical alterations by interactions between its principal constituents which affect the organoleptic, nutritional, and technological properties of heated milk [8]. For example, the Maillard reaction of milk can result in the event of a time-temperature failure upon sterilization process. Another example of the major problem that resulted from heating temperature can be cited: it is the fouling of heat exchanger surfaces by denatured and aggregated milk proteins. Indeed, fouling not only decreases heat transfer efficiency but also impacts the economic performance of a manufacturing facility resulting in the deterioration of the quality of the resulting dairy product can be spoiled because milk cannot entirely be pasteurized [5]. Although thermal technologies remain widely used, the development of novel thermal techniques to preserve dairy products, including ohmic heating treatment (OHT), microwave, and radiofrequency heating technologies, continues to attract significant industrial and scientific interest. These methods share a key characteristic: heat is produced immediately upon the process, leading to notable improvements in both energy use and heating efficiency.

2.1 Ohmic treatment

Ohmic treatment (OT) is commonly used to pasteurize food products. In this thermal process, electrical resistance produces heat. The ohmic effect is observed by conducting electricity effectively, as milk contains sufficient free water with dissolved ionic salts [4, 9]. OT has recently attracted attention due to its ability to produce products of higher-quality compared to those processed using conventional methods. In fact, OT allows for the application of higher pasteurization temperatures, leading to longer shelf life, while preventing denaturation of milk proteins due to the rapid heating rates employed [5, 10]. Furthermore, OT presents other major benefits including the minimization of issues with surface fouling or thermal damage to dairy products when compared to conventional heating, the lowest heat losses and maintenance costs, and finally its environmental friendliness [4]. For all these reasons, ohmic heating is currently garnering growing interest within the dairy industry [5]. However, this technology presents some drawbacks including its high initial costs and limited validation protocols. Despite these disadvantages, it offers a significant advantage for milk processing by preventing overheating and reducing surface fouling, which helps preserve thermosensitive compounds.

2.2 Infrared heat treatment

Infrared heat treatment has been widely used as a thermal process in the food industry including dairy in order to preserve foods (pasteurization and dishydration) and to cook them (frying). This technology has shown great efficiency in food pasteurization as it destroys both bacteria and spores yeast and even molds regardless of whether the food is liquid or solid. However, the efficacy of this technology in reducing microbial load on various parameters including types of microorganisms and moisture content in food [5]. For dairy products, infrared treatment is currently used in order to inactivate pathogenic bacteria leading to a longer shelf life and better quality characteristics. For instance, infrared heat treatment was shown to effectively reduce pathogenic bacteria in milk, including *Staphylococcus aureus*, with its concentration decreasing from 0.10 to 8.41 log₁₀ cfu/mL following the treatment [4, 11]. However, despite these notable advantages, the use of infrared treatment is still reduced in the dairy industry. Therefore, further research into the potential effect on sensory and quality changes in milk during this technology could provide insights into the effectiveness of this method and potentially introduce a new pasteurization technique for the dairy industry [11].

2.3 Microwave processing

Microwave technology is regarded as an innovative thermal treatment method that has seen growing use in recent years, both in the food industry and for domestic purposes. Household microwaves usually operate at a frequency of 2.45 GHz, while industrial microwave systems operate within a frequency range of 915 MHz to 2.45 GHz. A key advantage of microwaves is their ability to generate heat instantly, which significantly shortens processing time and lowers operational costs compared to conventional dry-heating methods [12].

Continuous-flow microwave showed a significant impact on the quality of milk such as the denaturation of the main whey protein β -lactoglobulin, and the inactivation of milk enzymes including alkaline phosphatase and lactoperoxidase [13].

Furthermore, this technology has been shown to be effective for milk pasteurization, with inactivation curves for microorganisms closely resembling those achieved with traditional heating methods [5]. For instance, microwave treatment showed a significant efficiency for inactivating *L. monocytogenes* at 71.7°C for 10 minutes [14]. In some cases, this technology was even a UHT treatment for fluid milk. However, microwave treatment technology may exhibit major disadvantages. This includes potential issues such as uneven heating and the occurrence of cold spots leading to a risk safety of the food. Furthermore, microwave technology faces challenges in process control and high energy consumption. Additionally, the impact of heating on the dielectric properties of food is not fully understood, requiring process validation for each dairy product individually [15].

3. Novel nonthermal processing technologies

Nonthermal technologies have proven effective in inactivating microbes while maintaining the functionality and nutritional quality of milk and dairy products. Moreover, these technologies could pave the way for the development of innovative and nutritious products within the dairy sector [7]. According to the literature, novel nonthermal technologies in dairy industry include high-pressure processing (HPP), ultraviolet, irradiation, ultrasounds and pulsed electric field technologies [4, 5].

3.1 High-pressure processing

High-pressure processing (HPP) is a nonthermal food preservation technique designed to achieve microbial inactivation similar to thermal methods while preserving nutritional and sensory qualities [16]. It is regarded as a promising alternative for milk pasteurization and sterilization, as it inactivates microorganisms and certain enzymes under pressures that range between 100 and 1000 MPa [17]. Beyond its antimicrobial effects, research has explored HPP's ability to induce reversible and irreversible changes in treated milk. Several comprehensive reviews discuss its impact on milk's physicochemical and nutritional properties [17–19]. HPP primarily affects milk's components by causing structural alterations, which depend on pressurization conditions such as pressure and duration [4]. This technique offers several advantages including preservation of dairy products with similar characteristics to those present before processing, Shelf life similar to thermal pasteurization, and homogeneity of treatments as the pressure is uniformly applied throughout the food product [5]. However, this process is unable to destroy spores without including heat treatments. Furthermore, research in HPP processing revealed that this technology may alter some physical and chemical properties of milk [20]. Therefore, it is reported that this technology can be used in the treatment of raw milk prior to the production of yogurt and cheese [21].

3.2 Pulsed electric field processing

Pulsed electric field technology (PEFT) is a novel processing technology that has demonstrated the ability to partially inactivate microorganisms in a sublethal manner, affect specific enzymes, and help retain or restore the physicochemical and functional properties of raw dairy products, especially when compared to traditional thermal treatments. Starting with PEFT, it is distinguished by its great potential for

successfully extending the shelf-life of milk and dairy products, as it is highly effective for liquids, utilizing voltage pulses ranging from 25 to 80 kV/cm [22]. In addition to its strong ability to inactivate microorganisms, this method has minimal impact on the flavor, nutritional, and functional properties of milk [23]. For instance, Bendicho et al. [24] investigated the contents of fat-soluble vitamins as well as water-soluble vitamins in milk as a function of PEFT treatments. These authors noted that PEFT technology reduced the concentration of ascorbic acid in milk without any significant impact on the other studied vitamins. Meanwhile, Xiang et al. [25] reported that milk proteins were denatured by a rate of 25% upon PEFT treatment. Thus, PEFT technology may exhibit significant impact on the technological properties of treated milk, including its ability to be transformed into cheese and yogurts.

3.3 Membrane filtration techniques

Membrane filtration has been utilized in the dairy industry since the 1960s, separating compounds by retaining some as retentate and allowing the others to pass through as permeate. The liquid product is pushed through a membrane using hydrostatic pressure or transmembrane pressure. Membranes are defined by their pore size and cutoff point, with common types including ultrafiltration, microfiltration, nanofiltration, and reverse osmosis [26]. The main difference between these techniques is the size of the membranes. For example, microfiltration allows particles ranging from 0.2 to 2 μm to pass through the membrane, operating at relatively low pressures, while ultrafiltration uses semipermeable membranes that only permit water and small molecular compounds to pass, with a cutoff threshold of 10,000 MW (molecular weight). On the other hand, reverse osmosis operates under high-pressure and only allows low molecular weight solvents to permeate through the membrane. Finally, nanofiltration, which can be viewed as a form of reverse osmosis, permits only monovalent ions to pass, with a cutoff of 100 MW. The substance that is blocked by the membrane is called “retentate” or “concentrate,” while the portion that passes through is referred to as “permeate” [7]. Membrane filtration techniques, particularly macrofiltration, have been shown to effectively remove microorganisms and spores, as demonstrated by numerous studies. These methods are also employed in the fractionation of dairy proteins and the reduction of enzymatic activity in milk [27]. Additionally, the use of membranes in the dairy industry has contributed to the production of higher-quality products with improved sensory attributes. However, some researchers caution against relying solely on microfiltration for microbial safety, as certain spores and pathogenic bacteria may survive and proliferate during the shelf life of products like milk [7]. Moreover, the similar size of fat globules and microbes can lead to rapid fouling, resulting in reduced system performance [27].

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Thermal and Nonthermal Processing of Dairy Products

B.S. Ashoksuraj, B.O. Madhu and Shanmugasundram Saravanan

Abstract

Thermal and nonthermal methods are essential in ensuring the safety, quality, and extended shelf life of dairy products. Thermal processing involves the application of heat to destroy harmful microorganisms and extend the shelf life of dairy products, such as pasteurization and sterilization. Pasteurization is done to eliminate pathogens while preserving the taste and nutritional value. Whereas sterilization requires applying higher temperatures, ensuring the destruction of all microorganisms, and allowing for a long shelf life without refrigeration. Nonthermal processing, which preserves nutritional and sensory qualities without significant heat, includes techniques like high-pressure processing, ultraviolet treatment, microfiltration, pulsed electric field processing, and ultrasound processing. Both methods, with their own advantages, find their applications within the dairy industry for maintaining the safety, quality, and longevity of dairy products.

Keywords: pasteurization, sterilization, cold plasma, high-pressure processing (HPP), ultra-high temperature (UHT), pulsed electric field (PEF), ultrasound, UV, ozone, microwave, irradiation

1. Introduction

This chapter explores various processing techniques for dairy products, discusses the advent of technology and its adaptation in the industries, and ensures the safety, quality, and longevity of milk and its derivatives. These sophisticated processes are broadly categorized into thermal and nonthermal methods. Thermal processing, a well-established technique, harnesses the power of heat to eliminate harmful microorganisms, enhance shelf life, and improve certain product characteristics. Techniques such as pasteurization, ultra-high temperature (UHT) processing, sterilization, and evaporation or drying are pivotal in this regard, each contributing uniquely to the stability and safety of dairy products.

On the other hand, nonthermal processing represents a frontier in dairy innovation, utilizing advanced methods to preserve the nutritional and sensory qualities of products without significant heat application. High-pressure processing (HPP),

ultraviolet (UV) treatment, microfiltration, pulsed electric field (PEF) processing, and ultrasound processing are some of the prominent nonthermal techniques that achieve microbial inactivation and enzymatic control while retaining the inherent freshness and nutritional integrity of dairy products.

The interplay of these thermal and nonthermal methods forms the backbone of modern dairy processing, ensuring that consumers receive products that are not only safe and long-lasting but also rich in flavor and nutrition. This chapter delves into the principles, applications, and benefits of both thermal and nonthermal processing techniques, with emphasis on the nonthermal techniques offering a comprehensive overview of their roles in the dairy industry and also discusses the future emerging technologies in dairy processing. Through this exploration, we aim to provide a thorough understanding of how these methods complement each other to enhance the quality and safety of dairy products.

2. Thermal processing of dairy product

Thermal processing involves the application of heat to destroy harmful microorganisms and extend the shelf life of dairy products, such as pasteurization and sterilization. Pasteurization is done by heating milk to 72°C (161°F) for 15 seconds (HTST) or 63°C (145°F) for 30 minutes (LTLT) to eliminate pathogens while preserving the taste and nutritional value. Whereas applying higher temperatures, such as UHT (ultra-high temperature) processing, where milk is heated to 135°C (275°F) for 2–4 seconds, is termed sterilization. These methods are widely followed in the dairy processing industry to ensure the destruction of all microorganisms and allow for long shelf life without refrigeration.

2.1 Ultra-high temperature processing

Ultra-high temperature (UHT) processing has been introduced over other thermal techniques due to its ability to sterilize milk at high temperatures for a short time, preserving its flavor and nutrients and extending shelf life. UHT produces sterile milk by processing it at high temperatures ranging from 135°C to 150°C for brief periods, likely within 10 seconds. UHT milk can be stored at room temperature for approximately 6 months when packaged aseptically [1]. Specifically, processing the milk for 2–3 seconds at 135–145°C can destroy pathogenic spore-forming microbes and also increase the shelf life for several months without the need for refrigeration [2]. *Geobacillus*, a thermophile, and spore-forming bacterium, is the primary target of the process, which aims to reduce the initial microflora by nine logarithmic units. There are multiple steps in the UHT process: preheat, high heat, cooling, homogenization, and aseptic or sterile packaging.

UHT systems in industries can be direct or indirect. In the first, there is direct contact between the product and the heating medium. The product is heated and cooled quickly. In the second system, heat is transferred to the product through a heat exchanger rather than the product and heating medium coming into direct contact. To maintain the flavor profile, direct heating is better than indirect heating, which is considered a severe treatment [3].

UHT developed cooked and sulfur-based flavors due to severe processing temperatures compared to pasteurized milk. Upon storage for months, UHT-processed milk can develop undesirable browning, fat separation, formation of sediment, and gelation.

All these physiochemical changes were due to Maillard, proteolytic, oxidative, and lipolytic reactions caused by the high temperature during processing [4]. A study was conducted to investigate the physicochemical changes that occur during the processing and storage of UHT milk. Milk with 3% fat and 8.5% solid not fat was processed at 140°C (4 seconds), and their properties were assessed for 4 months. A decrease in pH during the storage of UHT milk was observed, which resulted from the lactose breakdown, proteolysis, dephosphorylation of casein micelles, and calcium phosphate precipitation. There was an increase in viscosity during the storage of UHT milk, which is directly linked to proteolysis, which can lead to gelation and, consequently, a rise in viscosity. The rise in sedimentation value during storage of the sample was observed, which was attributed to the aggregation of proteins or the clustering of protein particles of different sizes. Colloidal Ca and Mg and lightness values were observed to be decreased [1].

The degradation of vitamins during processing and storage is a concern, as it can lead to reduced nutritional value in UHT milk. High temperature has little impact on fat-soluble vitamins, but water-soluble vitamins may be partially degraded during UHT processing. This processing method results in a reduction of B vitamins by 10%, folic acid by 15%, and vitamin C by 25%. The effects on the nutritional quality of proteins, minerals, and fats are minimal, and any changes are influenced by factors such as storage conditions, oxygen levels, and the type of packaging used [5]. UHT processing does not eliminate aflatoxin M1 (AFM1) contamination in milk. While UHT processing can reduce microbial contamination and extend shelf life, it is not effective in destroying aflatoxins, which are heat-stable toxins [6].

UHT processing of milk results in notable changes to its metabolomic composition. During the treatment, lipase hydrolysis of milk fat leads to an increase in fatty acids such as octadecanoic and hexadecanoic acid. Lactose undergoes hydrolysis, leading to higher concentrations of glucose and galactose, as well as the formation of lactulose, a marker used to identify UHT processing. The high temperatures involved in UHT processing also accelerate the Maillard reaction, producing furosine, a specific marker of heat intensity. Additionally, certain nutrients, including dodecanoic acid, are diminished, potentially reducing the milk's nutritional value [7]. A major disadvantage of UHT processing is its impact on sensory properties, particularly the flavor and texture of the milk, which may become altered due to the high temperatures involved.

The ability of UHT to extend shelf life and reduce food waste contributes to more efficient resource use and lowers the environmental impact of milk production. Though it involves high temperatures, the short processing time requires minimal energy compared to conventional thermal processing [8]. Since thermal treatments negatively impact the color, texture, and nutritional value of milk, there is a growing need for emerging technologies that can increase shelf life without lowering product quality. The implementation of such novel techniques may offer the dairy industry a long-term solution, a balance between preservation and preserving the nutritional and sensory qualities of dairy-based products.

3. Nonthermal processing of dairy products

Thermal processing is widely used in the food industry as an effective method for microbial inactivation, ensuring food safety and extending shelf life. However, this technique often results in undesirable changes to the nutritional and sensory

properties of food. Moreover, it poses sustainability challenges due to its high energy consumption and substantial water usage, making it less environmentally friendly [9]. These drawbacks have spurred significant interest among food researchers to explore innovative processing methods that achieve effective microbial reduction while preserving the nutritional and sensory qualities of food. Emerging technologies offer promising alternatives, particularly in maintaining the quality of dairy products, as they operate at lower temperatures and require shorter processing times compared to conventional thermal methods. Cold plasma (CP), high hydrostatic pressure (HHP), pulsed electric fields (PEF), pulsed light (PL), ultraviolet (UV) treatment, ultrasound (US), and irradiation are widely recognized as advanced nonthermal processing techniques utilized in the dairy industry [10, 11]. These techniques are well-known for their effectiveness in microbial inactivation (**Tables 1 and 2**). However, they also have several other noteworthy applications, including enzyme inactivation, reduction of mycotoxins such as aflatoxins, and improvements in the functional and physicochemical properties of dairy products. Both their microbial inactivation capabilities and these additional applications are discussed in detail in the following sections.

3.1 Cold plasma as a processing technology for dairy products

Cold plasma (CP) technology is an effective antimicrobial approach that can effectively eliminate pathogens, enhancing the safety of dairy products [42]. This technique has been effective against various microbes and applied to different food matrixes in dairy products. As a green technology, CP is cost-effective and ensures sterilization at low temperatures without leaving any residues in the food system. Additionally, it preserves the nutritional and sensory properties of dairy products. The minimal impact on product quality, green technology, compliance with ecological standards, and high sterilization efficiency make it a preferred choice for enhancing microbial safety in dairy products [12].

Processing techniques	Treatment condition	Food sample	Effect on microbial and physicochemical property	Reference
Cold plasma	Indirect dielectric barrier discharge (DBD) plasma treatment was applied for 15 minutes	Raw cow milk	<ul style="list-style-type: none"> The sharp decline in the microbial population. pH increased to 6.85, with a decrease in titratable acid (TA) and total soluble solids (TSS). 	[12–15]
	Atmospheric cold plasma in the form of spark discharge (SD) and glow discharge was applied for up to 30 minutes	Raw cow milk	<ul style="list-style-type: none"> α-Lactalbumin and ceasing intensity reduction in SDS-PAGE. The antigenicity of β-lactoglobulin increased and casein and α-lactalbumin were decreased. Changes in secondary structures (β-turn and β-sheets). Decrease in amino acid compositions. 	[16–21]

Processing techniques	Treatment condition	Food sample	Effect on microbial and physiochemical property	Reference
High-pressure processing	HPP was applied at 600 MPa for 10 minutes	Fresh cow milk	<ul style="list-style-type: none"> • A 100% reduction in total plate count and 100% in other specific species. • Consistent pH and titratable acidity were observed. • Retained all vitamins and minerals, though some degradation happened while storage. 	[22–25]
	A pressure of 500 to 700 MPa was applied for 10 minutes at 25°C	Yogurt	<ul style="list-style-type: none"> • Gel firmness was increased with pressure, maximum value found in 700 MPa • An increase in viscosity was observed in 600 and 700 MPa. • Treatment decreased the wheying-off from milk. • Increased color, flavor, and taste were observed. 	[26]
	Sample was treated at 400 MPa for 15 minutes	Standardized, pasteurized, and homogenized cow milk was pretreated before cheese-making	<ul style="list-style-type: none"> • Increase in soluble N, proteolysis process enhanced by HPP pretreatment. • ACE inhibitory activity was increased. • Higher DPPH radical scavenging and antioxidant activity. 	[27]
Pulsed electric field processing	Sample was treated with 20 pulses under an electric field strength of 24 kV/cm	Raw milk	<ul style="list-style-type: none"> • No changes in amino acid composition, β-lactoglobulin, and fatty acid content. • A 0.13 log was observed in PEF-treated samples. 	[28]
	An electric field strength of 20–40 kV/cm was applied for 5–13 μ s.	Raw goat milk	<ul style="list-style-type: none"> • A log reduction of 3.87 was done for <i>Escherichia coli</i>. • β-Carotene, riboflavin, niacin, and thiamine were minimally degraded. 	[29]
	UV-C treatment was applied with a fluence of 1.3–15.0 J/cm ²	Ricotta cheese	<ul style="list-style-type: none"> • Off-odor developed when fluence was increased more than 3.1 J/cm² • Small protein particles were formed, lipids interaction, decreased solubility, S-S enhancement, and secondary structure changes were observed. 	[30]

Processing techniques	Treatment condition	Food sample	Effect on microbial and physiochemical property	Reference
Ultrasound processing	Ultrasound with a frequency of 20 kHz and an intensity of 10–50 W was applied for up to 30 minutes at 70°C	Full-fat milk	<ul style="list-style-type: none"> • Increase in temperature leads to protein denaturation. • Acid gelation happens at 30 W (50°C) • Fat globules reduced at treatment more than 30 W and above 50°C. • The specific surface area of fat has been increased. 	[31]
	Ultrasound was applied using a 13 mm diameter probe at energy densities of 0.3, 0.9, 1.8, 2.4, and 3.0 kJ/cm ³ , with a maximum temperature of 42°C	Chocolate milk beverage	<ul style="list-style-type: none"> • Smaller-sized fat globules were distributed. • Changes in ACE inhibitory activity, antioxidant activity, fatty acid, and volatile profile. • Reduced nutrient losses. 	[32]
Membrane filtration	Membrane filtration was performed using a membrane with a pore size of 1.4 µm at 50°C	Skim milk	<ul style="list-style-type: none"> • All somatic cells were retained. • Residual fat was removed. 	[33]
	Microfiltration (MF) was performed using a membrane with a pore size of 0.14 µm	Skim milk for the production of cheese	<ul style="list-style-type: none"> • Casein was separated and concentrated in retentates. • Lactose, minerals, and serum protein were depleted. 	[34]

Table 1. Impact of non-thermal processing technique on microbiological, nutritional, and sensory characteristics.

Processing techniques	Advantages	Limitation	Reference
Ultra-high temperature processing	<ul style="list-style-type: none"> • Prolonged shelf-life • Reduced energy expenses and operational cost 	<ul style="list-style-type: none"> • Sulfury and cooked flavor. • Separation of fat, irreversible changes in protein structure and denaturation, storage gelation, lactose isomerization and Maillard reaction were negative impacts of UHT. 	[35–37]
Microwave processing	<ul style="list-style-type: none"> • Temperature irregularities in milk during heating, particularly in cold zones, raise concerns about microbial safety 	<ul style="list-style-type: none"> • Continuous-flow MWH offers fast heating and prevents fouling, making it promising for milk treatment. • Challenges include process control difficulties and high energy costs. 	[38]
Ohmic heating processing	<ul style="list-style-type: none"> • Immediate process starts and stops capabilities. • High energy conversion efficiency of 90%. • Reduced fouling, leading to lower plant cleaning costs. 	<ul style="list-style-type: none"> • Denatured whey protein deposits on electrode surfaces heighten the risk of microbial contamination and decrease operational efficiency. • Electrode corrosion occurs due to the presence of hydroxyl radicals (OH). • Fouling builds up on the electrode surfaces. 	[38]

Processing techniques	Advantages	Limitation	Reference
Cold plasma processing	<ul style="list-style-type: none"> • Minimal heat-induced alterations due to the nonthermal process. • Rapid processing time. • Energy-efficient technology. • Preserves the sensory quality of the products. • Effective antimicrobial action. • Limited structural changes. • Resource-efficient technology. • Environmentally safe technology. • No compromise on quality. 	<ul style="list-style-type: none"> • High-cost. • Limited penetration depth. • Accelerated lipid oxidation in high-fat products. • Potential for protein modification and flavor. 	[39]
High-pressure processing	<ul style="list-style-type: none"> • Preserves flavor and nutrient content without the need for preservatives or additives. • Uniform treatment across different masses and processing times. • Nonthermal destruction of microorganisms. • Extended shelf life. • Clean technology with a flexible system for various products and operations. • Ability to modify texture. • Reduced thermal degradation. 	<ul style="list-style-type: none"> • High initial investment and maintenance costs. • Spore destruction is not guaranteed without thermal treatment. • Lack of industrial-scale equipment for large-scale processing. • Water is required for compression. • Pressure-induced protein denaturation. 	[39]
Pulsed electric field processing	<ul style="list-style-type: none"> • Minimal processing required. • Shorter treatment time. • Retention of color, flavor, and nutrients. • Suitable for batch or continuous processes. • Improved mass transfer. • No chemical reactions involved. • Reduced fouling. 	<ul style="list-style-type: none"> • High initial investment needed. • Applicable only to certain food types. • Limited effectiveness against spores. • Inability to inactivate enzymes. • Bubble formation decreases efficiency. 	[39]
Pulsed light processing	<ul style="list-style-type: none"> • Absence of leftover compounds and no use of chemicals, disinfectants, or preservatives. • Greater penetration depth and stronger emission power. • Minimal energy cost. • Effectively controls oxidative reactions due to the brief pulse duration. 	<ul style="list-style-type: none"> • Its effectiveness in controlling food heating is limited. • A shadow effect has also been observed. 	[40, 41]

Processing techniques	Advantages	Limitation	Reference
Ultrasound processing	<ul style="list-style-type: none"> • A non-thermal method with various uses. • Enhanced transfer of mass. • Minimal chemical alterations. • Low operational expenses. • Energy-saving. 	<ul style="list-style-type: none"> • Industrial-scale processing equipment is still unavailable. • Concerns about the potential health effects of free radicals produced. • Certain food items may develop undesirable flavors or physico-chemical defects. 	[39]
Irradiation	<ul style="list-style-type: none"> • Cold process • Decreased reliance on preservatives. • Lower risk of pest migration in agricultural products. • Eco-friendly. 	<ul style="list-style-type: none"> • Negative consumer perceptions. • Concerns about radiolytic by-products and free radicals. • Significant initial investment required. • Not all food types are compatible with irradiation. • Potential loss of nutrients such as vitamins and minerals. 	[39]

Table 2.
Advantages and limitations of processing techniques in processing of milk.

Plasma is an electrically energized, ionized gas composed of ions, free electrons, free radicals, and atoms in their ground and excited states. The main active agents that give plasma its microbial inactivation capabilities are reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are produced when plasma is released into open-air atmospheres. These charged particles and neutral molecules make up plasma, a quasi-neutral ionized gas that is a very reactive and useful tool in food safety applications [13, 14]. Among various plasmas used for food processing, corona discharges and dielectric barrier discharges (DBD) are the two most often used for the processing of milk. DBD contains a pair of electrodes, and one or more dielectric barriers are used to prevent transition in arc formation. On the other hand, a corona discharge is a weak luminous discharge that typically occurs at atmospheric pressure close to sharp electrode structures (thin wires, points, or edges) [13]. The efficiency of CP is majorly dependent on the generation of ultraviolet light, the production of ROS, namely hydrogen peroxide, singlet oxygen, ozone, hydroxyl, and peroxy radicals, and ROS such as peroxy nitrite, peroxy nitrous acid, and nitric oxide [43].

The modes of microbial inactivation differ significantly between gram-negative and gram-positive microorganisms due to structural differences in their cell walls [12]. Majorly, CP has been used widely for microbial inactivation, enzyme deactivation, and mycotoxin control [44]. A key effect of CP is the disruption of the cell surface, leading to intracellular genetic material damage followed by cell lysis. Numerous studies have demonstrated its potential in microbial inactivation with different mechanisms within dairy matrixes, showcasing its effectiveness in reducing pathogens while preserving the quality and nutritional value of the products.

A recent study has focused on sheep milk processing by CP to study the effect of microbial reduction and also physiochemical characteristics and protein structure. Sheep milk was treated for 30, 180, and 300 seconds with DBD-CP. The performance of the treatment was compared with raw and pasteurized milk. The inactivation rate (IR) was increased from 15.15% to 78.5% as the treatment time increased,

significantly almost matching the IR of pasteurization. Also, reactive species produced during the treatment reduced the pH level of milk. The Fourier transform infrared (FTIR) spectra revealed significant changes in the secondary structure of proteins in raw milk after processing. Notably, cold plasma (CP) samples exhibited only slight modifications compared to pasteurized samples, which underwent major structural changes due to protein unfolding and denaturation during thermal processing. This highlights that cold plasma processing induces milder alterations in protein structure, preserving its integrity more effectively than traditional thermal methods [14].

High voltage atmospheric cold plasma (HVA-CP) has demonstrated effectiveness in reducing aflatoxin M1 (AFM1) in milk, achieving a degradation rate of 65.0% with air and 78.9% with 65% oxygen. The reduction of AFM1 increased as the treatment time was extended, with a more than 60% decrease after a 20-minute HVACP treatment, lowering the AFM1 concentration in skim milk from an average of 1 µg/L to a safe level of 0.5 µg/L. While there was a slight change in pH, the milk's color remained largely unaffected. These findings highlight the potential of cold plasma as a promising method for reducing AFM1 levels in milk without altering its visual properties [16].

Many studies have also shown that cold plasma treatment in dairy products has certain drawbacks, including accelerating lipid oxidation and degrading sensory qualities. For example, the impact of DBD-CP on liquid milk was investigated, and reported a significant increase in lipid peroxidation [17]. The control sample showed the lowest value compared to ultra-high temperature (UHT) sterilized, pasteurized, and CP treated. The values have been increased as voltage and treatment time increased. Excessive processing time could lead to accelerated lipid oxidation. Potential remedies for the problem of lipid oxidation include adding antioxidants to food before CP treatment, reducing the amount of time food is exposed to CP, using a lower voltage during treatment, lowering the oxygen content of the carrier gas, and improving the CP application procedure before its use in food items [18].

Furthermore, in addition to its effectiveness in microbial inactivation, toxin degradation, and minimal alterations of physiochemical properties, cold plasma treatment stands out as a sustainable approach. By extending shelf life and reducing spoilage, particularly in highly perishable dairy products, CP contributes to economic sustainability. The process utilizes renewable resources such as electricity and generates non-toxic by-products, promoting environmentally responsible practices. CP can reduce energy consumption by up to 50% compared to conventional pasteurization, positioning it as a highly efficient and sustainable food safety solution. But currently, CP is not yet commercially utilized for the processing industry, as further extensive research and optimization are required for successful scaling up [19, 20].

3.2 High-pressure processing of dairy products

High-pressure processing (HPP), a well-known alternative technique to thermal processing, has been used for various food applications, namely, microbial reduction, enzyme inactivation, shelf life enhancement, and physiochemical modification [22]. HPP for milk was first demonstrated in 1899 by Hite, who significantly reduced the bacterial count when treated at 680 MPa for 10 minutes (27°C). Despite this early discovery, HPP was not widely applied to milk preservation for almost 90 years. In recent decades, however, there has been growing interest in leveraging HPP for milk and dairy product manufacturing, primarily as an alternative to traditional pasteurization methods [45].

HPP involves subjecting food to extremely high pressures, up to 6000 times atmospheric pressure, typically between 300 and 700 MPa. At pressures exceeding 400 MPa, this method is highly effective in inactivating most vegetative bacteria. One of the key factors behind the global acceptance of HPP is its ability to process foods uniformly, regardless of their size, shape, or mass. The effectiveness of HPP is governed by two fundamental principles. According to Le Chatelier's Principle, any chemical reaction, phase transition, or molecular configuration change that reduces volume is accelerated under high pressure. Additionally, the Isostatic Principle ensures that pressure is transmitted uniformly and instantaneously throughout the food, regardless of its size, shape, or geometry [23].

The high pressure used in this technique majorly aids in microbial inactivation, disruption of casein micelles, protein denaturation, and solubilization of micelle-associated minerals in milk. Microbial inactivation by HPP happens through various mechanisms, namely, cell wall and membrane disruption usually happens above 400 MPa, inhibition of protein synthesis, disruption of ribosomes, and inactivation of intracellular enzymes [46]. There was a major reduction in bacterial count, like 100% in specific bacteria like *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, and 75% in total plate count. HPP did not change the fat content, as the high pressure does not affect the fat globules, and lipolysis is inhibited. Also, the protein and solid content were not affected by HPP. Significant changes in milk vitamins were observed despite the low vitamin levels in milk, except for a slight increase in vitamin C [24].

In addition to preserving the nutritional attributes, HPP has been shown to enhance the sensory properties of milk, contributing to improved flavor, texture, and overall consumer appeal. Milk samples were treated for 10 minutes at 600 MPa, which has been compared with thermally processed samples. Superior organoleptic qualities in color, mouthfeel, milkiness, and aftertaste were shown by HPP-processed milk; increasing creaminess could increase its general acceptability and preference [25].

In addition to microbial activation, HPP was used in the cheddar cheese-making process to improve the functional properties of cheese. The results showed that the HPP of Cheddar cheese decreased residual rennet activity and minimized the breakdown of intact casein levels. This reduction in rennet activity contributed to lower proteolysis, which affected the cheese's texture and the impact on insoluble calcium content and protein cross-linking. However, over the ripening period, the slower proteolysis in HPP-treated Cheddar appeared to counterbalance the initial negative effects on textural properties like hardness, helping to preserve the overall quality of the cheese [47].

HPP has also been employed on micellar casein concentrate (LMCC) to enhance microbial quality. LMCC is highly perishable as the membrane used in microfiltration will retain the bacterial spores and cells. Conventional thermal processing can adversely affect the sensory qualities of food, including its taste, aroma, and texture. Research has examined the combination of HPP and nisin addition on LMCC stability. The results revealed that while HPP caused color changes, these changes were more evident at the high-pressure range. Notably, HPP treatment at 450 MPa and nisin effectively limited the microbial count for up to 7 days at 10°C, showcasing its potential to enhance microbial safety and maintain product quality.

Despite its widespread application, a major limitation of the process is the use of pressure-transmitting fluid, as the entire process relies on the compression of this fluid, which can introduce potential contamination and add operational complexity. Protein-rich foods may experience visible deterioration in appearance

due to pressure-induced denaturation [23]. One major challenge associated with this technology is the high capital expenditure and operational cost of establishing pressure-based techniques. This technique has been commercialized for fruit juice at 500–600 MPa; however, its application in milk remains at the research level and has not been widely commercialized. However, it offers a low environmental impact when compared to thermal processing, mainly due to its low power consumption [22]. Despite its disadvantages, HPP relies on simple physical processes, making it a clean-label option for food manufacturers.

3.3 Pulsed electric field processing of dairy products

In the dairy industry, pulsed electric field (PEF) technology is a promising, sustainable, nonthermal method that, like all techniques, can reduce the negative effects of traditional thermal processing without compromising the nutritional, functional, and sensory qualities of milk and milk products. Unlike other methods, PEF is exclusively effective for liquids, as it requires the medium to flow through a treatment chamber where short pulses of high-voltage electric fields are applied [48]. Electric pulses applied to cells and materials generate a strong electric field that can affect cell membranes, a process known as electroporation. This phenomenon creates pores on the nanoscale in the lipid bilayer of the cell membrane, allowing molecules and ions to pass through that would normally be unable to cross. When cells are exposed to intense electric fields, their permeability increases, leading to electroporation. If a sufficient transmembrane voltage is applied for an adequate duration, irreversible pores form on the membrane, causing cell death. Electroporation happens when pulses disrupt the charge balance in cell membranes, creating a localized electric field. If the pulse intensity exceeds a certain threshold, nanopores form in the lipid bilayers, allowing disruptive molecular transfer that compromises normal cellular functions. The extent of electroporation depends on the pulse's magnitude and duration, with milder pulses potentially restoring function and more intense pulses triggering apoptosis [49]. The efficiency of electroporation is majorly dependent on pulse duration, intensity, frequency, number of pulses, and also the property of the cell focused on.

A recent study compared the effect of PEF on whey protein content and bacterial viability with conventional methods like UHT processing, LTLT pasteurization, and microfiltration (MF). PEF treatment at 24 kV/cm with 25 μ s pulses resulted in bacterial and coliform counts of 0.9 and 2.43, respectively, without affecting undenatured whey protein (4.98 mg/mL). In contrast, conventional pasteurization methods inactivated bacteria but reduced whey protein content: UHT treatment eliminated β -lactoglobulin, LTLT caused a 75% reduction, and MF showed the lowest β -lactoglobulin levels, with reductions of 10% and 27% for LTLT and UHT, respectively [28].

In addition to microbial inactivation, PEF treatment at lower and moderate intensities can stimulate bacterial proliferation and enhance metabolic activity by temporarily altering cell permeability. This reversible electroporation, which causes only temporary damage to the bacterial cell, can promote fermentation by encouraging more efficient microbial growth and activity. One such study has been conducted to explore the low-intensity PEF as pretreatment for a starter culture mix consisting of skim milk, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* prior to fermentation of yogurt. In comparison to traditional yogurt processing (CY) (~4.7 hours), the majority of PEF treatments shortened the fermentation time by 0.31–0.52 hours. The sample pre-treated for 400 μ s at 1 kV/cm and 150 Hz with 8- μ s pulses resulted

in short fermentation time (~4.18 hours). Immediately following processing and throughout refrigeration storage, the physicochemical and sensory properties of the PEF-treated yogurt were comparable to those of the CY [50]. Compared to microorganisms, enzymes are more resistant to PEF treatment. This can be seen as a benefit because the PEF process can kill only microorganisms while maintaining the activity of certain beneficial enzymes that are used in dairy processing [51].

PEF treatment shows significant promise as an alternative to traditional heat pasteurization, offering benefits such as minimal changes to sensory profiles and excellent nutrient retention, resulting in a fresh-like character for dairy products. However, up-scaling PEF to commercial levels remains a challenge due to differences in process uniformity and conditions between small-scale and industrial equipment. Additionally, the higher capital costs of PEF compared to traditional thermal pasteurization must be justified by a premium-priced product, making PEF particularly suitable for high-value dairy items like liquid infant food, bioactive dairy products, fruit-dairy blends, and cheeses made from raw milk [51]. However, PEF is not yet commercially implemented for milk processing, as it remains at the research and pilot scale.

3.4 Pulsed light technology for dairy products

The pulsed light (PL) technology uses short, intense pulses of broad-spectrum “white light” that span the ultraviolet to the near-infrared spectrum. Often, a few flashes are enough to significantly inactivate the microbes. The ultraviolet portion of the light has photochemical effects that cause mutations in microbial DNA, resulting in genetic damage, impaired transcription, and replication, which ultimately leads to cell death. This is the main cause of the germicidal effect. Furthermore, photothermal effects may also occur during pulsed light treatment, contributing to microbial inactivation. PL treatment with a fluence of 0.26–26.25 J/cm² can efficiently destroy natural microorganisms and enzymes, namely, alkaline phosphatase. Maximum fluence showed 94% inactivation of enzyme and 3.2 log reduction in total plate count, with a rise in temperature up to 55°C [52]. Specific microorganisms like *Staphylococcus aureus* in milk [53], *Cronobacter sakazakii* in dry milk [54], *Escherichia coli* in goat milk [55], and *Listeria monocytogenes* in sliced Mortadella [56] are inactivated by PL at optimum conditions.

The sensory characteristics of intense PL-treated reconstituted milk (RM) and milk powders (MP) made from those samples were more influenced by the PL exposure duration than by the initial temperature. The intensity of the burned aroma, umami taste, and overall aroma in both milk powders increased with the length of light exposure and milk that has been reconstituted. In RM alone, rising temperatures intensified the scents of sulfur and animals. Compared to the control MP and the control RM, all exposure times resulted in higher levels of light-induced flavors and aromas, including cardboard, sulfur, and brothy flavors, among others. The only extreme processing condition that changed the milk powder’s appearance in comparison to the control was a 4-pass exposure at a temperature of 25°C. Pulsed light (PL) treatment does not impact the amino acid composition, induce conformational changes in proteins, or generate oxidative products, making it a promising nonthermal technology for preserving the nutritional and structural integrity of food components [40].

While PL is effective in reducing microorganisms in foods, it may cause certain quality alterations, such as increased lipid oxidation and color changes, which can influence consumer acceptance [56]. Its limited penetration depth restricts its

effectiveness on the surface of food products. Additionally, the high initial cost of PL equipment may limit its accessibility for smaller food producers. Regulatory requirements and thorough safety assessments are also essential to ensure that pulsed light-treated foods meet all health and safety standards [57]. These challenges underscore the need for careful optimization of PL treatment to balance microbial safety, product quality, and economic feasibility.

3.5 Ultraviolet light technology for dairy processing

The increasing demand for safe and sustainable food products has led to a significant increase in interest in ultraviolet (UV) light as a nonthermal solution in the food industry. Its germicidal qualities are very effective in eliminating a variety of microbial pathogens, including bacteria, viruses, fungi, yeasts, and molds, especially when combined with shortwave UV light. Food quality is maintained while the negative effects of thermal processing are reduced thanks to UV processing, which is an inexpensive and energy-efficient substitute for conventional heat treatments [58].

In the electromagnetic spectrum (EM), between visible light and X-rays, there is a non-ionizing source of invisible light called UV. It spans wavelengths from 100 to 400 nm and is categorized into four main types: UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm). UV light, typically at a wavelength of 254 nm, is particularly effective in killing microorganisms [59]. The European Food Safety Authority (EFSA) confirmed that UV-treated milk, which undergoes UV radiation after pasteurization to extend shelf life and boost vitamin D3 levels, is safe for consumption under the specified conditions [58]. UV dose, exposure time, UV transmittance, and turbulence of liquid food decide the efficiency of disinfection. A recent study investigated the impact of UV-C light on the inactivation of microorganisms, along with the amino acid (AA) profile, functional group, and other physiochemical characteristics of cow milk. At the dose of 4.6 J/cm², *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia coli* were inactivated by 4.92, 4.41, and 4.07, respectively, in cow milk. NO significant changes in AA profile, functional group, metabolites, and sensory attributes [60]. Other than liquid milk, various milk powders (MP), namely whole MP [61] and goat MP [62] have been effectively sterilized by UV-C treatment.

Pulsed UV (PUV) light offers a novel approach for modifying the allergenic properties of cow's milk proteins. The effects of different PUV light doses (ranging from 0.1 to 10 J/cm²) were evaluated on both commercial cow's milk proteins (such as casein, α -lactalbumin, and β -lactoglobulin) and reconstituted milk proteins. The study measured the degree of hydrolysis (DH), changes in protein profiles using SDS-PAGE, and antigenicity levels. Results showed that at higher fluences, DH increased in both commercial and reconstituted milk proteins. SDS-PAGE analysis revealed a reduction in band intensity for casein and β -lactoglobulin at 5 and 10 J/cm², but α -lactalbumin remained unaffected. Additionally, antigenicity testing showed a significant reduction in casein (24%) and β -lactoglobulin (47%) at a fluence of 10 J/cm². Currently, it is not commercially adopted due to scalability and penetration limitations in liquids.

3.6 Ultrasound processing of dairy products

Ultrasound (US) is a novel technology that has been widely used in the dairy industry to improve the functional qualities of milk and dairy products and increase

process efficiency. It has demonstrated potential advantages in emulsification, milk homogenization, lactose/fat crystallization, altering the functional and physical characteristics of dairy products, degassing, fat separation, and foaming when used as a substitute or adjunct to traditional treatments [63]. US is the term used to describe sound waves with frequencies higher than the human hearing threshold (~20 kHz). The medium is compressed and expanded (rarefied) by the alternating low- and high-pressure cycles produced by ultrasound. Cavitation bubbles are tiny vacuum bubbles produced by negative pressure during rarefaction. Over many compression/rarefaction cycles, these bubbles enlarge until they are unable to absorb energy anymore, which results in a collapse that emits energy. Acoustic cavitation, or implosion, is the term used to describe this bubble formation, growth, and implosion process. High temperatures (5000 K) and pressures of 500 atm produced by cavitation bubbles have the potential to produce extremely high shear forces. Physical and chemical effects in the liquid, including microstreaming, agitation, shock waves, micro-jetting, turbulence radical generation, sonoluminescence, etc., are caused by the violent collapse of a cavitation bubble [64].

Ultrasound in the food industry can be divided into two categories according to intensity: low-intensity ultrasound (LIU), which has intensities below 3 W/cm², and high-intensity ultrasound (HIU), which has intensities greater than 3 W/cm². They can also be divided into three groups based on power and frequency: (i) medium-power medium-frequency (100 kHz–1 MHz), (ii) low-power high-frequency (1–100 MHz), and (iii) high-power low-frequency (20–100 kHz) [65].

In addition to its role in bacterial inactivation, ultrasound (US) offers numerous promising applications in the dairy industry. US homogenization was applied before pasteurization to evaluate its effects on milk properties. US homogenization produced milk with a whiter appearance, with L* values of 99.62 ± 0.05, compared to 95.72 ± 0.30 in raw milk. This whitening effect occurs due to the reduction in fat globule size, which enhances light scattering. The more significant decrease in fat globule size observed with ultrasonic treatment, compared to conventional milk, also led to a more stable emulsion, maintaining the same level of stability over time. The authors claim that the shear forces produced by cavitation reduced the viscosity by breaking the oil into smaller pieces and improving the distribution of the proteins on the system's new interfaces. The zeta potential (ζ) was raised, and the creaming index and droplet size were reduced by the ultrasound treatment used to produce the emulsion [66].

In this regard, ultrasonication cavitation has become more and more popular in a variety of quality and dairy product processing fields. It has been applied to non-destructive quality testing, homogenization, milk component modification, protein allergy reduction, microbial destruction, enzymatic inactivation, fermentation, cheese and yogurt quality improvement, and lactose crystallization. With considerable processing cost savings, the ultrasonic energy needed for the mentioned applications is minimal and reasonably simple to scale up the production [65]. To consolidate the use in the large-scale production of the dairy industry, more research is still needed on the industrial and continuous processes that the US can use.

3.7 Ozone technology for dairy processing

Ozone has attracted significant interest in the food industry due to its strong oxidizing capabilities and effective antimicrobial properties. As consumer demand for a wide range of food products, from fresh to processed items with longer shelf

lives, increases, the potential for ozone application has grown. In 1997, ozone was recognized as a generally recognized as safe (GRAS) substance and was later classified as a food additive by the US FDA in 2001. When an oxygen molecule and an oxygen free radical combine to form triatomic oxygen, ozone is created [67]. The resulting ozone molecule is extremely unstable in both the gaseous and aqueous phases. Ozone subsequently breaks down into different reactive oxygen species (ROS), including oxygen free radicals, as a result of this structural instability. When ozone interacts with bacterial cells, it primarily targets the cell wall and membrane, which are composed of phospholipids. Ozone reacts with the polyunsaturated fatty acids in these phospholipids, causing peroxidation and forming ozonides that break down into lipid peroxides. This process increases permeability, leading to leakage of cellular contents, degradation, and eventual cell lysis. While ozone affects both gram-positive and gram-negative bacteria, it causes more significant damage to gram-positive species [68].

The dairy industry uses ozone for many reasons, including surface decontamination, soil removal from processing surfaces, microbial safety, etc. In the study, 400 mg/h of ozone was bubbled, and the microbial properties of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* were assessed. Within 20 minutes of ozone treatment, microbial reduction below 1 log CFU was noted for pathogens like *S. typhimurium* and *E. coli* [69].

Ozone technology increased the volume mean diameter of MC while decreasing those of WC. Concentrates treated with ozone had lower firmness and consistency values than the control groups. OT reduced MC's cohesiveness. OT reduced the samples' yellowness while increasing their lightness. OT was successful in lowering the microbial load in both MC and WC samples [70]. The economic feasibility of ozonation systems remains a limitation for their widespread use. While the technology requires relatively low operating costs, the high initial setup expenses discourage small-scale entrepreneurs. Additionally, a lack of consumer awareness about ozone technology contributes to the hesitancy in adopting ozonated products [68]. This processing is still under research and development for milk processing, and while it is commercially available for water sanitation and surface sterilization in dairy processing, its direct treatment of milk is not yet widely adopted commercially.

3.8 Irradiation processing of dairy products

Irradiation is a nonthermal technology that has sparked both significant interest and debate regarding its use in food processing over the years. While it offers potential for microbial control, its adoption in dairy products has been slow, mainly due to the established effectiveness of heat pasteurization in eliminating pathogens. Despite this, irradiation remains an area of exploration for improving food safety and extending shelf life without the need for high temperatures [71]. Irradiation uses high-energy ionizing radiation, such as X-rays, gamma rays, or electron beams, to treat food. When these radiation sources interact with food, they excite and ionize the components, causing molecular changes. Excitation makes living cells more sensitive to external factors, while ionization breaks down macromolecules into free radicals. These radicals disrupt the microbial cell division process by inhibiting DNA synthesis, ultimately preventing microbial growth. This method not only helps control pathogens but also extends the shelf life of food products [72].

The electron beam (e-beam) emitter targets food with electrons, inactivating microorganisms through direct and indirect mechanisms. Directly, ionizing radiation

damages DNA, preventing cell division. Indirectly, the e-beam generates reactive hydroxyl radicals that accumulate, causing DNA damage, physical harm, and ultimately cell lysis. Gamma (γ) radiation also works on a similar principle [73]. While e-beam irradiation is known for its microbial inactivation properties, it is intriguing that it also preserves or enhances the antitumor, antioxidant, and antidiabetic activities of cow milk fractions. In that way, a study explored the effects of e-beam irradiation at varying doses (5–20 kGy) on defatted cow milk fractions, focusing on its potential antidiabetic, antiproliferative, and antioxidant activities. Cow milk fractions treated with e-beam showed no cytotoxicity on HEK293T cells, except for sweet whey at 20 kGy and β -casein at 10 and 20 kGy. Irradiated β -casein exhibited anti-proliferative effects against A549 tumor cells. Whey fractions displayed strong α -amylase inhibition (73.24–8.99%) and retained or improved DPPH radical scavenging activity (6.27–59.75%) after treatment. Moreover, e-beam irradiation either improved or maintained the α -amylase inhibitory activity and antioxidant properties of both cow milk fractions [74].

In addition to improving the bacteriological quality of milk [75], gamma radiation also enhances the shelf life of butter by reducing microbial growth and spoilage. A slower rise in bacterial count was observed in butter made from irradiated fermented sheep cream compared to the control butter during storage [76]. Irradiation has limited use in dairy products due to concerns about its effect on organoleptic qualities. While accepted in some countries, its widespread adoption is slow and controversial [71]. Further research on consumer attitudes, process standardization, and feasibility is needed, along with education on its benefits for preserving and extending the shelf life of dairy products.

4. Emerging technologies in processing dairy products

4.1 Microwave heating

In contrast to traditional heating techniques like hot water, steam, hot gas, and so forth, MW heating is a new and alternative thermal technology that has been researched to guarantee the safety and extend the shelf life of milk and dairy products as well as to retain the quality of dairy products [77]. The preservation effects of MW processing have generally been attributed to their thermal impacts, which are primarily caused by an increase in temperature. However, other mechanisms may also play a major role, including (i) Targeted heating of microbial cells to a temperature higher than that of the surrounding fluid, resulting in quicker and more intense cell destruction; (ii) electroporation, where the electric potential applied across the cell membrane creates pores, leading to membrane rupture and leakage of cellular contents, significantly affecting processes like extraction and dehydration; and (iii) the influence of magnetic fields, which interact with essential cellular components like proteins or DNA, amplifying the destructive effect [78].

Numerous investigations have examined microwave technology as a substitute for traditional milk pasteurization as a processing method. A study demonstrated the use of MW heating and compared the results with conventional pasteurization techniques like LTLT, HTST, and UHT. A 5-log reduction of *Staphylococcus aureus* and *Escherichia coli*. The reduction of antimicrobial proteins, as noted in this study, was comparable to the reductions seen with UHT processing. When assessing the impact of microwave processing conditions against traditional heat treatments, no benefits

were found in terms of preserving antimicrobial proteins when compared to HTST and LTLT [79]. Another study effectively showcased the significant potential of the new system for continuous microwave heating of food products that are sensitive to fouling. Reconstituted skim milk concentrates with dry matter ranging from 27 to 36 g/100 g were processed using MW and tubular heat exchangers at temperatures ranging from 110°C to 125°C with a 5-second holding time to investigate heat-induced product changes and compare the results. Microwave heating led to a notable reduction in whey protein denaturation (35–40% less β -lactoglobulin denaturation and α -La) and less deposit buildup on the heating section, with only moderate to minimal color changes in the product after heating. Turbulent flow conditions with $Re > 3000$ inhibited significant aggregate formation for both heating methods. Future studies will focus on the microwave heating of native dairy concentrates, particularly in feasibility studies involving extended durations and higher heating temperatures (above 100°C) [80].

For dairy processing, microwave (MW) heating shows considerable potential, offering benefits such as reduced protein denaturation and minimal color changes. However, its adoption is hindered by the lack of sufficient experimental data for accurate modeling, energy consumption analysis, and cost evaluation. Additionally, managing heat leakage requires specialized technical expertise, and the high initial investment is a barrier to widespread implementation [77]. Overcoming these limitations through further research and technological advancements will be crucial to unlocking the full potential of MW heating in dairy processing.

4.2 Ohmic heating

Ohmic heating (OH) is an innovative thermal processing method that uses alternating current to heat food products. In this technique, electrodes are inserted into the food, and the electric current is passed through the product, causing it to heat up. OH has gained attention in the dairy industry due to its potential for rapid and uniform heating, which can help preserve the quality and nutritional value of dairy products while minimizing thermal damage. This method offers advantages over conventional heating by providing more controlled temperature increases, making it particularly suitable for heat-sensitive dairy applications [81]. A recent study investigated ohmic heating as an alternative to conventional pasteurization systems. Probiotic chocolate-flavored dairy dessert was prepared using milk subjected to ohmic heating at various electric field strengths (60 Hz), specifically 12 to 24 V/cm. OH-treated samples at 24 V/cm exhibited lower antioxidant activity, darker color, and reduced probiotic survival. The “bitter” taste was less pronounced in OH at 20 V/cm. Higher electric fields (20 and 24 V/cm) showed similar or even better results than pasteurization for most parameters. Sensory analysis linked OH-treated samples to lumps and fluidity, while pasteurized ones had “creamy” and “firm” textures. Volatile compounds like propanal and ethyl propanoate were common across treatments, with ethyl acetate unique to OH at 24 V/cm [82].

The advantages of OH include more uniform heat distribution compared to conventional heating and the potential for using clean energy sources, making it environmentally friendly. Additionally, despite its quick processing time, OH offers lower energy consumption than traditional pasteurization [83]. However, the higher initial costs, reduced effectiveness for fat-rich foods due to low conductivity, and less heat exposure for bacteria inside fat granules, along with the increased temperature leading to higher electrical conductivity and electrode corrosion, make it challenging for industrial applications [84].

5. Conclusion


This chapter explored thermal and nonthermal processing techniques in dairy processing, emphasizing their impact on safety, quality, and shelf life. While conventional thermal methods like pasteurization and UHT ensure microbial stability, novel thermal techniques such as MW heating and OH offer greater energy efficiency and reduced nutrient loss. Nonthermal technologies, including HPP, CP, PEF, US, PL, and UV, provide sustainable alternatives that preserve nutritional and sensory attributes while extending shelf life. Techniques offer a more sustainable alternative to traditional thermal methods by reducing energy consumption, minimizing water usage, and preserving the nutritional and sensory qualities of dairy products. Their effectiveness, however, depends on the specific characteristics of the dairy product, including its composition, safety requirements, and desired shelf life. As consumer demand for minimally processed and high-quality dairy products grows, nonthermal technologies present a promising future for sustainable dairy processing. The integration of these techniques, either individually or in combination, can enhance efficiency while maintaining product integrity and environmental sustainability.

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Sustainable Milk Processing: Reducing Waste and Enhancing Efficiency

Aws N. Al-Tayawi, Hadid Sukmana and Szabolcs Kertész

Abstract

The dairy industry faces pressing challenges in sustainability, particularly in managing water resources, energy efficiency, and waste reduction. This study explores innovative strategies and technologies aimed at addressing these challenges, emphasizing water conservation, energy optimization, and the valorization of dairy by-products. Effective water management practices, including recycling and advanced treatment technologies, are discussed as essential for mitigating environmental impacts. Energy efficiency in milk processing is highlighted through the adoption of renewable energy sources, energy recycling methods, and process optimization techniques. Furthermore, waste minimization is addressed via circular economy approaches, transforming dairy waste streams into valuable products. Constructed wetlands, physico-chemical treatments, and biological processes are examined for their effectiveness in treating dairy wastewater. The findings underscore the need for integrating advanced technologies and sustainable practices to enhance operational efficiency and environmental stewardship in the dairy industry. This holistic approach not only reduces the environmental footprint but also contributes to economic viability, paving the way for a more sustainable future in milk processing.

Keywords: dairy, water conservation, energy reduction, waste minimization, by-product utilization

1. Introduction

The dairy industry occupies a pivotal position in global food production; however, it is confronted with substantial challenges in achieving sustainability, particularly concerning water conservation, energy efficiency, and waste management. The growing demand for dairy products is accompanied by an increasing environmental impact, necessitating urgent measures to address these issues. Milk processing, a fundamental component of the dairy industry, contributes significantly to environmental degradation through extensive water consumption (**Figure 1**), high energy usage, and substantial waste generation. The imperative for sustainable practices in milk

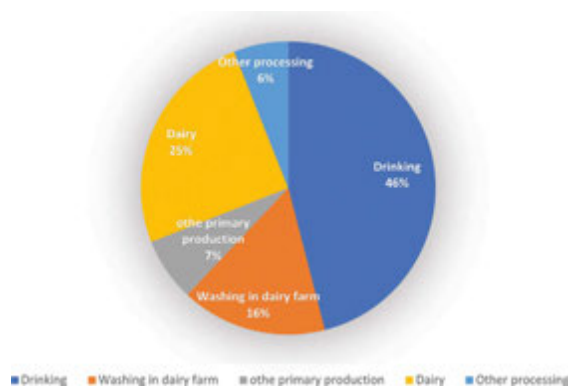


Figure 1.
Total freshwater consumption in skimmed milk production (L water/L milk).

processing has intensified in response to the expanding global population, mounting pressures on natural resources, and the industry's environmental footprint.

Water conservation remains a critical concern in dairy processing, where large volumes of water are employed for cleaning, processing, and packaging. It is estimated that for every liter of milk processed, the industry generates between 2.5 and 10 liters of wastewater, presenting significant environmental challenges if inadequately managed [1, 2]. The adoption of effective water management practices is essential not only for reducing wastewater volumes but also for ensuring the sustainability of water resources in dairy operations [3].

Energy efficiency constitutes another cornerstone of sustainable dairy processing. The energy-intensive nature of milk processing underscores the necessity of adopting innovative technologies and practices to lower energy consumption and greenhouse gas emissions [4]. Integrating renewable energy sources and implementing energy recycling methods can significantly improve the energy efficiency of dairy plants, fostering a more sustainable production model [5].

Furthermore, waste minimization and by-product utilization are indispensable elements of a sustainable dairy processing framework. The industry generates diverse waste streams, including whey and scum, which can be transformed into valuable products such as biodiesel or organic fertilizers [6, 7]. By embracing a circular economy approach, dairy processors can not only reduce waste but also generate additional revenue streams while contributing to environmental sustainability [8].

This chapter seeks to examine sustainable strategies in milk processing, emphasizing water conservation, energy reduction, waste minimization, and the effective utilization of by-products to enhance operational efficiency while mitigating environmental impacts.

2. Water conservation

The conservation of water in the dairy industry is vital due to its high consumption, which can reach up to 60 liters per kilogram of processed milk, largely attributed to cleaning and sanitation processes [9]. Addressing this challenge requires a combination of recycling, efficient management practices, and innovative technologies.

Implementing water recycling systems, such as recovering water from whey (a by-product of cheese production), has proven viable for cleaning-in-place systems, reducing freshwater demand and wastewater generation [10]. Additionally, membrane technologies like reverse osmosis can treat dairy wastewater, enabling its reuse within the plant, significantly lowering the total volume of freshwater used to produce dairy products which is known as 'water footprint' [11, 12]. Advanced cleaning technologies, such as nanofiltration, further enhance water conservation and sustainability in dairy processing [13]. Moreover, improving water management can cut water consumption and effluent bills by up to 30% [14]. Automated monitoring systems are particularly effective in identifying leaks and inefficiencies, replacing less reliable manual reporting methods [15]. The reuse of water from cooling systems for irrigation and investments in infrastructure maintenance can also mitigate waste [16]. Furthermore, adopting sustainable farming practices, such as using manure-based fertilizers and optimizing irrigation, supports groundwater recharge and reduces reliance on external water sources [17]. These measures also address nitrate leaching and its impact on groundwater quality, which is significant in intensive dairy farming [18].

On the one hand, emerging technologies, including artificial intelligence and machine learning, optimize water consumption predictions and management strategies, enabling more efficient resource use. Green technologies inspired by lean methodologies also streamline processes, reducing waste, including water usage [19]. On the other hand, with water scarcity, climate change, and a growing global population, sustainable water management in dairy production is more critical than ever. Efforts must focus on optimizing water use for sanitation, cow hydration, milk cooling, and mitigating heat stress, which collectively account for significant consumption [20]. **Figure 2** illustrates the sustainable water recycling in the dairy sector.

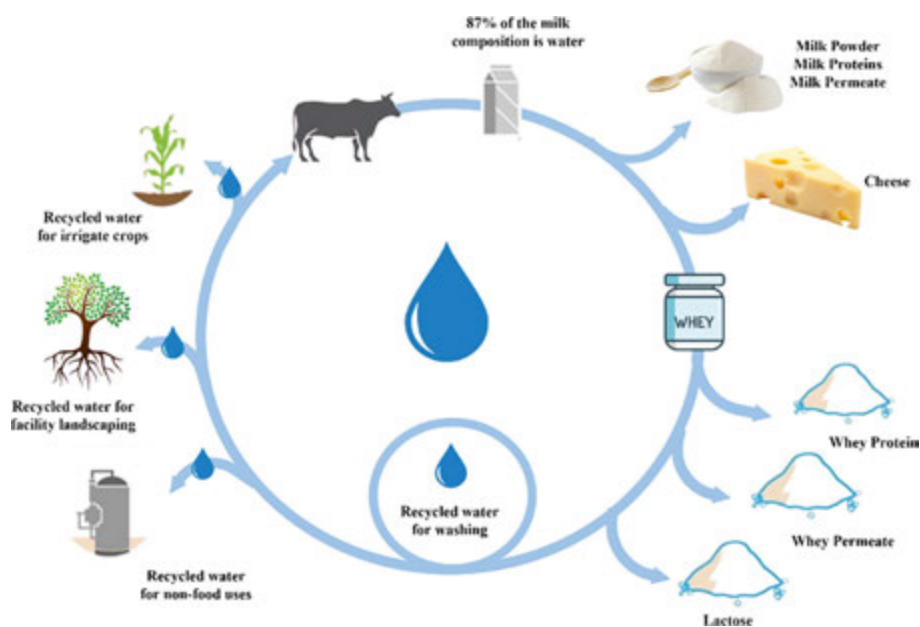


Figure 2.
Sustainable water conservation in dairy industry.

3. Energy efficiency and reduction

To sustainably reduce energy consumption in the dairy industry, several strategies can be implemented (**Figure 3**). Embracing renewable energy sources like solar power can minimize greenhouse gas emissions and reduce energy costs, contributing to sustainability [21]. Improving energy efficiency through upgraded equipment, optimized processes, and technologies such as membrane separation can significantly lower energy and water usage [22]. Implementing energy management systems, such as ISO 50001:2018, is an international standard that provides a framework for organizations to establish, implement, maintain, and improve an energy management system [23], and also integrating ISO 14001:2015 into the dairy industry can enhance energy efficiency and sustainability by promoting strategic environmental management [24]. Integrating Lean Six Sigma methodologies can optimize energy consumption and reduce the carbon footprint [25]. Addressing energy-intensive processes like pasteurization and homogenization during milk processing also offers substantial savings [26]. Utilizing dairy waste for bioenergy production through anaerobic digestion or fermentation can help the industry become more energy self-sufficient [8, 27]. Data-driven and AI-powered solutions can optimize energy use, improve feed management, and enhance sustainability [28]. Sustainable supply chain frameworks address environmental challenges and improve productivity while reducing input costs [29]. Finally, conducting life-cycle assessments and benchmarking environmental impacts can identify areas for improvement and guide sustainable practices [30–32]. These strategies collectively enable the dairy industry to reduce energy consumption, minimize environmental impact, and enhance sustainability for a more eco-friendly and cost-effective future.



Figure 3. Strategies for sustainable energy reduction in the dairy industry.

4. Waste minimization

Globally, over 60% of freshwater withdrawals are allocated to food production, with substantial volumes of wastewater being generated, particularly by the meat, dairy, and grain industries, which contribute approximately 24, 12, and 9%, respectively (**Figure 4**). Dairy wastewater is characterized by a relatively high concentration of soluble organic compounds, soluble and insoluble solids, and trace minerals. Initially, the pH is slightly alkaline (approximately 7.4); however, due to lactic fermentation, lactose in the wastewater is rapidly converted into lactic acid, resulting in an acidic pH shift to approximately 6.0. Additionally, dairy wastewater typically contains nitrogen levels ranging from 17 to 1120 mg/L and phosphorus concentrations from 10 to 500 mg/L [33].

In addition to implementing technologies that reduce water and energy consumption during milk processing, various other methods for waste minimization can be employed such as constructed wetlands, physico-chemical treatments, and biological treatments. Constructed wetlands include horizontal and vertical flows. Furthermore, physico-chemical treatments involve coagulation/flocculation, electro-coagulation, membrane separation, and adsorption. In addition, biological treatment includes anaerobic and aerobic methods (**Figure 5**).

4.1 Constructed wetlands (CWs)

Constructed wetlands (CWs) are regarded as a sustainable wastewater treatment approach and effective systems for treating municipal and agricultural wastewater, offering functionality comparable to conventional treatment systems while providing a low-cost, environmentally friendly, and energy-efficient alternative [34, 35]. The application of CWs presents an optimal solution for treating dairy wastewater, which contains high levels of organic and inorganic pollutants. This approach is both technically and economically advantageous, offering an effective means to mitigate the environmental impacts associated with dairy wastewater discharge [36, 37].

CWs are characterized by simple operation and maintenance requirements, as well as low installation costs, making them highly effective for removing organic

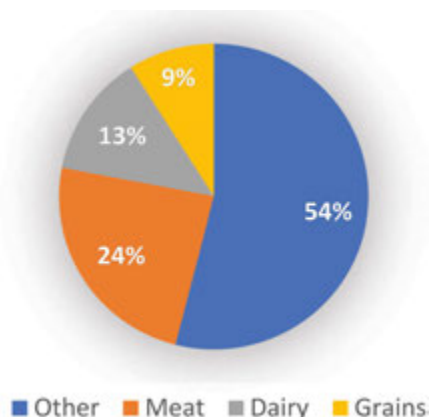


Figure 4.
Percentage contribution of food industries to wastewater production.

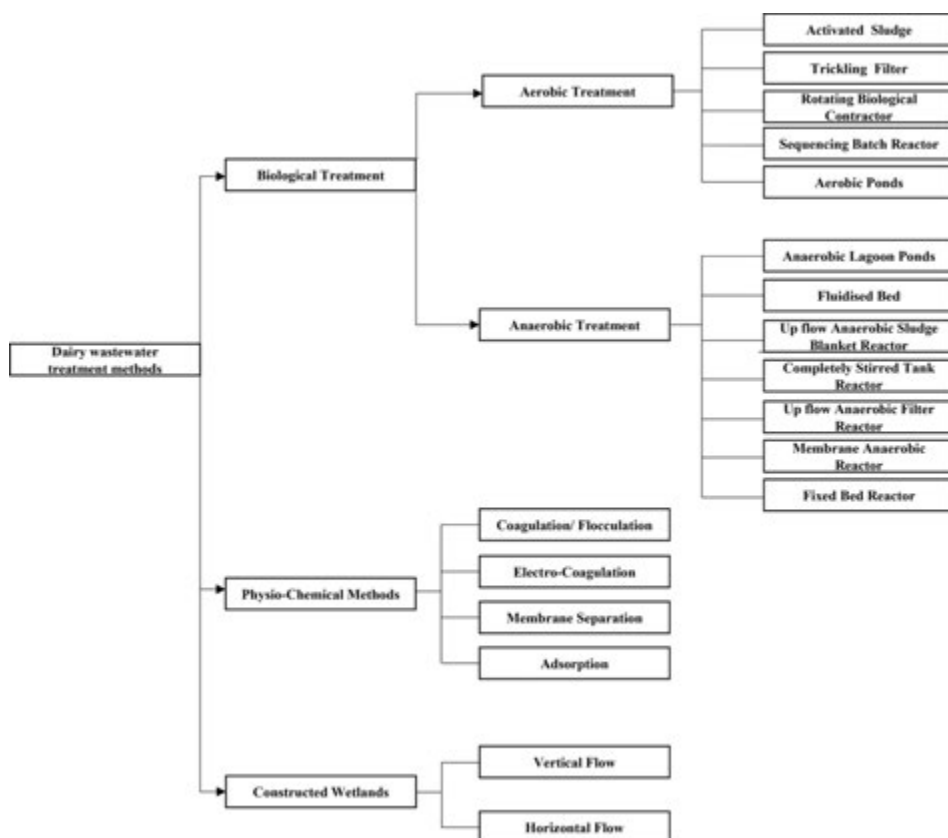


Figure 5.
Dairy wastewater treatment methods.

pollutants and nutrients such as biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), microorganisms, nitrogen, phosphorus, and trace elements [34, 38]. Their application contributes significantly to environmental protection and the sustainable management of wastewater, particularly suited for tropical developing countries [39].

Sub-surface constructed wetlands (CWs) can be classified into two types: sub-surface horizontal flow CWs (SSHF CWs) and sub-surface vertical flow CWs (SSVF CWs). In SSHF CWs, wastewater flows horizontally through the substrate material, facilitating treatment as it moves through the system. In contrast, SSVF CWs involve the intermittent dosing of wastewater onto the surface of sand and gravel filters. The wastewater percolates vertically through the filter media and is subsequently collected in a drainage system located at the base [40].

The efficiency in natural or constructed wetlands is influenced by factors such as wetland design, the composition of the microbial community, and the selection of plant species [41]. Therefore, plant species selection provides multiple advantages, playing a crucial role in creating conditions that directly or indirectly enhance the system's performance [42]. Additionally, the growth of vegetation and a variety of plant physiological processes are greatly impacted by environmental elements including air temperature and solar radiation. Air temperatures can encourage the growth of plants, which improves vegetative performance as well as bacterial growth and activity [43].

Several studies have conducted the effectiveness of CW treatment for dairy wastewater. For instance, the study was investigated by Gottschall et al., which used a constructed wetland system treating agricultural wastewater from a 150-cow dairy operation in Ontario, Canada. The research focused on evaluating the removal efficiency of total Kjeldahl nitrogen (TKN) and total phosphorus (TP). The system was dominated by the plant species *Typha latifolia* L. and *Typha angustifolia* L. In the first wetland cell, which received higher nutrient loading rates, plant uptake contributed to 0.7% of TKN removal. In contrast, in the second wetland cell, characterized by lower nutrient loading rates, plant uptake accounted for 9% of TKN removal, 21% of ammonium (NH₄⁺) removal, and 5% of TP removal [44].

The study carried out by Mohammed and Ismail used six horizontal sub-surface flow-constructed wetland (CW) microcosms to treat dairy wastewater. Five of the CW microcosms were planted with *Canna indica*, while one served as an unplanted control. The findings demonstrated significant treatment efficiencies, with chemical oxygen demand (COD) removal ranging from 89.92 to 98.2%. Additionally, high ammonium removal efficiencies were observed, ranging from 82.0 to 98.4%, and total suspended solids (TSS) removal efficiencies ranged from 93.4 to 99.2% [45].

The research by Sharma et al. evaluated the performance of a hybrid constructed wetland (CW) system, consisting of vertical-horizontal-vertical flow configurations, for treating dairy farm wastewater under the high-humidity climatic conditions of northern India. Tropical perennial plants such as *Arundo donax* L. were cultivated on both vertical flow beds, while *Hibiscus esculentus* L. and *Solanum melongena* L. were grown on the horizontal flow bed. The system demonstrated average removal efficiencies of $92.2 \pm 6.1\%$ for total suspended solids (TSS), $95 \pm 3.8\%$ for biological oxygen demand over 3 days (BOD₃), $83.6 \pm 9.0\%$ for total nitrogen (N), and $86.1 \pm 10.0\%$ for total phosphorus (P) in the horizontal flow bed. The average influent and effluent loads for BOD₃, total nitrogen, and total phosphorus were recorded as 7.0 ± 7.17 , 1.9 ± 0.7 , and $0.72 \pm 0.5 \text{ g m}^{-2} \text{ day}^{-1}$ and 0.3 ± 0.2 , 0.3 ± 0.2 , and $0.04 \pm 0.01 \text{ g m}^{-2} \text{ day}^{-1}$, respectively [46].

4.2 Physico-chemical treatment methods

Physico-chemical treatment methods are widely employed for the degradation and removal of protein colloids and milk fat in dairy wastewater. The management of fat, oil, and grease (FOG) is particularly challenging in facilities producing unskimmed milk, as well as in processes such as milk and whey separation, cheese and butter production, and milk bottling. Conversely, skimmed milk production typically generates fewer issues in this regard [47]. A combination of physico-chemical methods, including coagulation-flocculation, adsorption, and membrane processes, is commonly utilized for wastewater treatment in the dairy industry. Chemical precipitation and coagulation processes often employ precipitating agents such as aluminum sulfate, ferric chloride, and ferrous sulfate, which interact with milk components, thereby impacting the efficiency of these methods [48]. The coagulation-flocculation (CF) is one of the most efficient and extensively used techniques for industrial wastewater treatment due to its simple process and high performance [35, 49]. This process plays a pivotal role in the removal of natural organic matter, particulates, metals, inorganic ions, microorganisms, and other pollutants that contribute to turbidity, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) in wastewater [50, 51].

CF treatment involves the destabilization of suspended solids and colloids in dairy wastewater by introducing electrolytes, facilitating their aggregation into larger flocs

for easier removal [33]. Therefore, several factors of treated water including pH, conductivity, total dissolved solids (TDS), and COD levels are the main determinants of coagulant efficiency [50]. The CF process is typically divided into two stages: coagulation and flocculation. Coagulation involves the addition of chemical coagulants, such as iron or aluminum compounds, to neutralize the stabilizing forces of suspended particles. This is succeeded by flocculation, in which destabilized particles aggregate into larger flocs that may be readily separated via gravity settling [52].

Several studies have highlighted the effectiveness of CF treatment for dairy wastewater. For instance, Ayeche observed that the use of residual lime as a coagulant reduced suspended solids (SS) by 92% and total phosphorus by 83% [53]. Another study by Suman et al. evaluated the use of water treatment sludge as a coagulant for synthetic dairy wastewater, achieving removal efficiencies of 93% for turbidity, 65% for COD, 67% for BOD, 84% for total suspended solids (TSS), and 85% for TDS under optimal conditions [54]. Furthermore, Mateus et al. reported the use of *Moringa oleifera* (MO) as a natural coagulant in combination with coagulation/flocculation/sedimentation (CFS) and microfiltration (MF) or nanofiltration (NF) processes. This integrated treatment approach achieved high removal efficiencies, including 96% for COD and 99% for turbidity and color [55].

4.3 Biological treatment methods

Biological treatment methods have demonstrated high efficiency in reducing the levels of organic matter in a variety of wastewater types. The adaptability of microbial systems enables biological treatment to accommodate diverse wastewater compositions. However, maintaining optimal physical conditions such as temperature, oxygen levels, and pH is critical to preserving the viability and functionality of microbial consortia [56].

Biological processes offer an economically feasible option for the removal of nitrogen and organic substances from wastewater. Advances in treatment designs continue to improve efficiency while reducing operational costs [57]. Therefore, biological treatments, including aerobic and anaerobic processes, are among the most widely preferred techniques for treating dairy wastewater globally [52, 56].

Aerobic treatment refers to the microbial decomposition and oxidation of organic substances in the presence of oxygen. However, aerobic processes generate substantial amounts of sludge, leading to costly disposal challenges, and require high energy inputs, which are a significant disadvantage. On the other hand, anaerobic treatment is especially appropriate for dairy wastewater because it has a high chemical oxygen demand (COD), substantial organic content, and warm temperature. Compared to aerobic methods, anaerobic processes do not require aeration, produce minimal sludge, and demand less space, making them advantageous for industrial-scale applications [58].

Innovative applications of biological treatment for dairy wastewater were investigated. For instance, research by Kusmayadi et al. demonstrated the integration of anaerobic digestion and microalgae cultivation for dairy wastewater treatment. Using *Chlorella sorokiniana* SU-1, high biomass production (3.2 ± 0.1 g/L) was achieved, along with removal efficiencies of $86.8 \pm 6\%$ for COD, $94.6 \pm 3\%$ for total phosphorus (TP), and $80.7 \pm 1\%$ for total nitrogen (TN). For high-COD dairy wastewater, an integrated anaerobic digestion and microalga phycoremediation process achieved biomass yields of 4.25 ± 0.10 g/L, with removal efficiencies exceeding 93% for COD, TP, and TN [59].

Similarly, the study by Das et al. applied biological treatment using bacterial and microalgal consortia through response surface methodology. Under optimal conditions (COD 720 mg/L, ammonium 55 mg/L, pH 7, mixed liquor suspended solids (MLSS) 1500 mg/L, and reaction time of 24 hours), COD and ammonium removal efficiencies were achieved at 98.61 and 97.42%, respectively. In a subsequent phase using a microalga consortium, nitrate nitrogen and phosphorus removal efficiencies reached 98.64 and 90.53%, respectively [60].

Furthermore, Custodio et al. investigated bacterial biomass isolated from eutrophic lake sediments for dairy wastewater treatment. Anaerobic processes effectively reduced with the highest average values of chlorides, sulfates, phosphates, and COD. Meanwhile, aerobic processes achieved the highest average values of turbidity, conductivity, ammonium, suspended solids, and biochemical oxygen demand (BOD). Anaerobic reactors predominantly contained bacterial orders such as Clostridiales (14.27%) and Lactobacillales (12.44%), while aerobic reactors were dominated by Burkholderiales (20.67%) [61].

Rivas et al. demonstrated the effectiveness of integrating coagulation-flocculation and aerobic biodegradation for treating cheese whey wastewater, achieving a significant reduction in the high organic load, as indicated by COD levels [62]. Similarly, Moulkaf evaluated the performance of a combined physico-chemical and biological treatment approach against a direct activated sludge process for dairy wastewater treatment, concluding that the combined method was superior in reducing COD and BOD levels [63]. Furthermore, Permana et al. investigated vermifiltration, a biological treatment approach, demonstrating its potential to effectively reduce COD, BOD, and total suspended solids in farm dairy effluent [64].

5. Sustainable future in dairy production

The dairy industry aims to ensure its long-term sustainable development. Achieving competitive advantages through technological advancements necessitates re-evaluating existing concepts and practices employed at the farm level, during processing, and in post-production, while integrating innovative strategies to address future challenges. The global dairy industry recognizes the need for innovative technologies to enhance resource utilization and is advancing scientific and technology solutions that promote environmentally sustainable and socially responsible dairy production [65]. To contribute effectively to global food security, the dairy sector must adopt a holistic approach to efficiently converting milk into value-added dairy ingredients and products while ensuring sustainability at every stage of production [66].

Future dairy farming enterprises are expected to become larger and incorporate lateral integration strategies to manage cattle classes more effectively within single operations. Automation, robotics, and advanced sensors will replace many labor-intensive tasks, facilitating improved sustainability and efficiency in dairy farm management [67]. The modernization of dairy production requires the extensive implementation of smart systems, where automation and digitization play pivotal roles. Technologies such as animal chipping, smart milking systems, fatness scanners, and advanced data systematization will enhance operational efficiency and drive the industry's progress [68].

Assessing the sustainability of dairy production systems requires a multidimensional approach that considers environmental, economic, and social factors [69]. Future sustainability initiatives should focus on conserving biodiversity and

improving ecosystem services by preserving natural habitats and integrating environmentally friendly farming practices [26]. Despite major improvements in productivity, the dairy industry's long-term viability depends on implementing sustainable breeding goals and management techniques, particularly from an agroecological aspect. To address future issues, high-production systems will need to be refined with a greater emphasis on environmental efficiency, livestock genetic variety, climate adaptability, animal health, and welfare [70]. Extending cow longevity by minimizing involuntary culling can enhance animal welfare, reduce healthcare costs, and increase lifetime profitability, thereby contributing to a more sustainable dairy sector. This approach also improves resource efficiency for dairy farmers by optimizing the overall utilization of available resources [71].

6. Conclusion

Sustainability in the dairy industry is a multifaceted challenge that requires innovative and integrated solutions. This study emphasizes the critical importance of water conservation, energy efficiency, and waste minimization in achieving sustainable milk processing. The implementation of advanced water recycling technologies, renewable energy systems, and by-product valorization offers substantial environmental and economic benefits. Constructed wetlands, physico-chemical methods, and biological treatments demonstrate significant potential for mitigating the environmental impacts of dairy wastewater. A circular economy approach, coupled with smart technologies such as artificial intelligence and data-driven optimization, enhances resource efficiency and promotes environmental sustainability. By adopting these strategies, the dairy sector can reduce its ecological footprint, improve productivity, and align with global sustainability goals.

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Chapter 4

Precision Fermentation: The Path to Animal-Identical Dairy Solutions

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Abstract

This chapter explores the transformative potential of precision fermentation in the production of animal-identical dairy proteins. As consumer demand for sustainable and ethical food sources rises, traditional dairy production faces challenges related to environmental impact and animal welfare. This chapter delves into the innovative technologies that harness microbial fermentation to create dairy components like whey protein, casein, and lactoferrin, replicating their nutritional and functional properties without relying on animal agriculture. By examining the scientific principles, biotechnological advancements, and potential applications of precision fermentation, this chapter aims to provide insights into how these methods can revolutionize the dairy industry and contribute to a more sustainable food system. Through case studies and future projections, it highlights the role of precision fermentation in meeting global dietary needs while reducing the ecological footprint of dairy production.

Keywords: animal-identical proteins, sustainable production, whey protein, casein, lactoferrin, synthetic biology, microbial fermentation, precision fermentation

1. Introduction

The global food system faces increasing pressure due to climate change, a rapidly growing population expected to exceed 9.8 billion by 2050, and the environmental impact of conventional agricultural practices [1]. Livestock farming alone accounts for at least 26% of global greenhouse gas (GHG) emissions, with half of these emissions stemming directly from livestock production [2]. This reality has intensified calls for sustainable alternatives that can address the rising demand for protein while mitigating the ecological impact of food production.

Simultaneously, dietary patterns are shifting as consumers grow more aware of the ethical, environmental, and health implications of animal-based foods. In regions like the UK, the percentage of vegetarians and vegans has increased significantly in recent years, accompanied by a steady decline in meat and dairy consumption [3]. These changes reflect broader global trends, with many consumers seeking to reduce their reliance on animal products due to concerns about sustainability, animal welfare, and environmental degradation.

Dairy products, a cornerstone of human diets for millennia, are central to these discussions. While traditional dairy provides essential nutrients like calcium, protein, and vitamins, its production is resource-intensive, raising serious concerns about its environmental impact and sustainability. This has created a pressing need for innovative solutions that preserve the benefits of dairy products while reducing their ecological footprint.

Precision fermentation (PF) has emerged as a transformative technology capable of addressing these challenges. Leveraging microorganisms to produce animal-identical proteins such as whey protein and lactoferrin, PF offers a revolutionary approach to creating dairy products without animals. Unlike plant-based alternatives, which often face criticism for differences in taste, texture, and nutritional content, PF-based products are indistinguishable from their animal-derived counterparts. This technology not only reduces the environmental impact of dairy production but also addresses growing consumer demand for sustainable and ethical food options.

The potential of PF extends beyond its ecological benefits. By enabling the production of bioidentical dairy proteins such as casein, it has the power to reshape the dairy industry, ensuring food security and aligning with consumer preferences for animal-free alternatives. While PF has long been used in other industries, such as pharmaceuticals for producing insulin and in food technology for enzymes such as microbial rennet [4], its application in dairy protein production represents a groundbreaking leap in sustainable food systems.

However, the path to widespread adoption is not without challenges. High production costs and regulatory complexities, particularly in regions like the European Union (EU), present significant barriers. Despite these obstacles, PF-based dairy products have already reached markets in the United States and Singapore, showcasing the viability of this innovative approach.

As the global food industry grapples with reconciling the nutritional demands of a growing population with the urgent need to address climate change and animal welfare concerns, precision fermentation emerges as a transformative solution. By producing bioidentical dairy proteins without animals, PF offers a sustainable, ethical, and innovative pathway to meet the challenges of modern food production.

This chapter explores the profound implications of precision fermentation for the dairy industry and beyond, examining its technological foundations, economic potential, and the opportunities and challenges it presents for the future of food.

2. Precision fermentation

Precision fermentation is an innovative biotechnological process that combines traditional fermentation techniques with modern advancements in biotechnology. It is designed to efficiently produce specific compounds of interest, such as proteins, vitamins, pigments, fats, or flavor molecules, by utilizing genetically engineered microorganisms as biological factories.

The Precision Fermentation Alliance (PFA) and Food Fermentation Europe (FFE) define precision fermentation as [5]:

"Precision fermentation combines the process of traditional fermentation with the latest advances in biotechnology to efficiently produce a compound of interest, such as a protein, flavor molecule, vitamin, pigment, or fat."

This process is distinct from traditional fermentation in its ability to produce highly targeted and complex compounds. In the food industry, precision fermentation has become particularly valuable for creating animal-identical proteins that mimic those found in dairy, meat, and other animal-based products, without the use of animals. Additionally, precision fermentation can produce human-identical breast milk proteins, offering innovative applications in infant nutrition.

By leveraging microorganisms like yeast, bacteria, microalgae, or (filamentous) fungi, precision fermentation enables the sustainable production of compounds that are essential for creating food products with the same taste, texture, and nutritional properties as conventional animal-derived foods. It offers a scalable, efficient, and environmentally friendly alternative to traditional methods of food production, positioning itself as a key technology in addressing global food security and sustainability challenges.

2.1 Process development

The precision fermentation process is a sophisticated and highly optimized sequence of steps that enables the efficient production of specific proteins, such as dairy proteins like casein and whey. These proteins are crucial for creating animal-free alternatives to milk, cheese, and yogurt. By leveraging advanced biotechnological techniques, precision fermentation allows for scalable and sustainable production of these proteins, mimicking the properties of traditional dairy products without relying on animal agriculture. The below-mentioned steps are done for the development of a precision fermentation-based product [6].

2.1.1 Selection of host organism

The selection of an appropriate host for precision fermentation is critical for ensuring the success of the fermentation process, the efficiency of production, and compliance with regulatory standards. Several criteria should be considered to identify the most suitable host organism, as outlined below:

2.1.1.1 Genetic manipulability

A host organism must be amenable to genetic modification to enable the insertion of target genes and optimize expression. Easy and precise genetic engineering ensures that the host can produce the desired product with high yield and functionality. Availability of genetic tools, robust transformation methods, and established genetic databases are key factors in this consideration.

2.1.1.2 Metabolic efficiency

The host's metabolic pathways should efficiently convert substrates into the desired product, particularly for non-protein-based products. High metabolic efficiency reduces resource waste and shortens production time. Hosts with simplified metabolic networks are often preferred, as they enable streamlined optimization of production processes.

2.1.1.3 Growth characteristics

Host organisms with fast growth rates, high cell densities, and high productivity are highly desirable. Robust growth characteristics can shorten production timelines

and enhance process economics. Key factors include tolerance to environmental stresses, such as temperature fluctuations, pH variations, and osmotic pressure, as well as the ability to perform effectively in industrial-scale bioreactors.

2.1.1.4 Reliable and efficient expression system

A host must possess a reliable expression system for the consistent production of the desired product. This includes strong and inducible promoters, efficient translation machinery, and mechanisms to facilitate proper folding and posttranslational modifications of the product when needed.

2.1.1.5 Food safety and regulatory considerations

The chosen host should be Generally Recognized as Safe (GRAS) or approved by relevant regulatory authorities. Ensuring food safety and compliance with regulatory frameworks is critical for the commercialization of precision fermentation products. Hosts with a history of safe use in food and pharmaceutical industries are often preferred.

2.1.1.6 Genetic stability

Stability in genetic and metabolic profiles ensures consistent performance across production cycles and minimizes the risk of mutations that could compromise yield or quality.

2.1.1.7 Growth on defined media

Industrial-scale fermentation often requires the use of defined media to ensure reproducibility and minimize costs. A suitable host should grow efficiently on cost-effective, defined media composed of readily available nutrients. This simplifies downstream purification and reduces contamination risks. In contrast, the use of complex media poses several disadvantages, including reduced repeatability due to variable compositions, increased risk of contamination from undefined components, and higher costs associated with sourcing of complex ingredients.

2.1.1.8 Downstream processing

The ease of product recovery and purification is heavily influenced by the host. Hosts that secrete the product into the culture medium or exhibit minimal interference with downstream processes are preferred. Reducing impurities and simplifying product isolation improve the overall process efficiency.

2.1.1.9 Scale-up potential

The selected host should be able to maintain its productivity and growth characteristics during scale-up from laboratory to industrial-scale bioreactors. Scalability requires consistent performance under varying conditions, including agitation, aeration, and nutrient supply.

2.1.2 Genetic engineering

Once the suitable host organism has been chosen, the gene encoding the target protein, such as casein or whey, is isolated from its natural source and engineered for optimal expression in the host. This phase involves several critical steps:

- *Codon optimization*: The gene's sequence is often optimized to match the codon preferences of the host organism. This optimization improves translation efficiency, ensuring that the host can produce the target protein at high levels. Codon optimization is particularly important when expressing complex proteins in microorganisms that may have different codon usage patterns.
- *Expression cassette design*: An expression cassette is designed, incorporating the necessary genetic elements to control and regulate protein production. This includes a promoter to drive gene expression and a terminator to signal the end of the transcription process. The expression cassette ensures that the gene is properly transcribed and translated into the desired protein within the host cell.
- *Secretion strategy*: One of the key decisions in gene expression engineering is whether the protein will be secreted by the host or retained inside the cells. Secretion is often preferred, as it allows the protein to be isolated more easily from the fermentation medium, reducing purification complexity. However, retaining the protein inside the cells can sometimes be more efficient, depending on the specific protein and the host organism's capabilities.
- This phase ensures that the host organism will produce the target protein in sufficient quantities while maintaining the protein's functionality and quality.
- *Metabolic engineering and optimization*: Metabolic engineering plays a crucial role in enhancing the efficiency of the precision fermentation process. By manipulating the metabolic pathways of the host organism, researchers can optimize protein production and improve yield. This process is often carried out in a "Design-Build-Test-Learn" (DBTL) cycle, an iterative framework for continuous improvement. The incorporation of artificial intelligence (AI) and machine learning (ML) has significantly accelerated the metabolic engineering process. These technologies help researchers optimize the DBTL cycle by enabling faster design iterations, improving strain development, and boosting protein yield efficiency.

2.1.3 Fermentation process design

The design of a precision fermentation process is critical to ensuring the efficient and scalable production of recombinant proteins, such as dairy proteins like casein and whey proteins. This process involves a series of decisions regarding the type of fermentation, induction methods, protein production strategies, and downstream recovery and purification systems. These elements are carefully optimized to maximize protein yield, minimize costs, and maintain product quality.

2.1.3.1 Types of fermentation

Fermentation processes are broadly classified into batch, fed-batch, and continuous modes, depending on how the culture medium is managed and the feeding strategy employed. Batch fermentation involves adding all ingredients, including the microorganism and nutrient-rich medium, at the start of the process. The fermentation runs to completion without further addition of nutrients, typically ending when the nutrients are exhausted, or the target product has accumulated to the desired level. This method is straightforward and easy to monitor, with a lower risk of contamination since no additional nutrients are added. However, it has drawbacks, including lower productivity once nutrients are depleted and limited scalability for industrial applications.

Fed-batch fermentation, on the other hand, begins with a small amount of nutrient medium, with additional nutrients added at regular intervals. This approach extends the production phase by continuous or intermittent supply of nutrients, leading to higher productivity. Fed-batch processes also allow for greater control over the environment, optimizing growth conditions for the organism. However, they are more complex, requiring careful monitoring of nutrient levels and feeding rates, and they pose a higher risk of contamination due to the ongoing addition of nutrients.

Continuous fermentation involves a constant supply of fresh nutrients while simultaneously removing fermentation broth at the same rate. This method supports continuous production of the target protein and allows for precise control over conditions, such as pH, temperature, and nutrient levels. While it offers the highest productivity, maintaining a steady-state culture can be challenging, and the process is more vulnerable to contamination, especially at industrial scales [7].

2.1.3.2 Induced vs. constitutive DNA for protein expression

Another key aspect of precision fermentation design is deciding how to introduce the gene encoding the target protein into the host organism. Induced expression involves a genetic construct that allows protein production only when a specific inducer molecule is added. This approach enables precise control over the timing and quantity of protein production, optimizing yield while reducing the metabolic burden on the host during early fermentation stages. However, induced expression can delay peak protein production and is susceptible to incomplete induction if the inducer is not properly administered.

Constitutive DNA, or non-induced expression, involves integrating the gene for the target protein into the host genome, enabling continuous protein production driven by constitutive promoters or natural regulatory systems. This method simplifies the process, as it eliminates the need for an inducer, but it can impose a constant metabolic burden on the host, potentially reducing overall productivity. Additionally, there is less flexibility in controlling the rate and timing of protein expression.

2.1.3.3 Extracellular vs. intracellular protein production

The choice between extracellular and intracellular protein production depends on factors, such as the protein's structure, solubility, and the host organism's capabilities. Extracellular protein production, common in yeasts and fungi, simplifies downstream recovery and purification by secreting the proteins into the culture medium. This approach often results in higher purity products, as secreted proteins are less likely to

be contaminated with cellular components. However, secretion bottlenecks can occur, and secreted proteins may undergo unwanted modifications that affect functionality.

Intracellular protein production involves accumulating proteins within the cells, often forming inclusion bodies. This method is particularly suitable for proteins that are difficult to secrete or require specific folding within the cell. While intracellular systems can produce proteins in high concentrations, extracting them requires cell lysis, which adds complexity and cost. Proteins produced intracellularly are also prone to aggregation, necessitating additional steps to refold them into their active forms.

2.1.3.4 Recovery and purification systems

Once the target protein is produced, it must be separated from the microbial culture and purified. Recovery and purification systems depend on whether the protein is produced extracellularly or intracellularly. Extracellular proteins, secreted into the culture medium, can be recovered using techniques, such as tangential flow filtration and chromatography. These methods facilitate the isolation of the target protein while removing cells and other impurities.

For intracellular proteins, cell lysis is necessary to release the proteins into solution. Lysis can be achieved through mechanical methods like high-pressure homogenization or bead milling, chemical methods involving detergents or osmotic shock, or enzymatic methods using cell wall-degrading enzymes. Following lysis, proteins are typically purified using filtration and chromatography, then concentrated and formulated to ensure purity and functionality.

2.2 Regulatory approval of precision fermentation-based dairy proteins

The regulatory approval process for precision-based dairy proteins is essential to ensure the safety, quality, and environmental sustainability of these innovative products. Produced through precision fermentation technologies, these genetically engineered proteins mimic animal-derived dairy proteins and hold immense potential to transform the food industry by offering sustainable, animal-free alternatives. However, before these products can be legally marketed and sold, they must meet rigorous safety standards and undergo thorough evaluations by regulatory agencies across different regions [8].

Regulatory agencies play a crucial role in assessing the safety of precision-based dairy proteins for human consumption. In the United States, the Food and Drug Administration (FDA) oversees the safety of food ingredients under the Federal Food, Drug, and Cosmetic Act (FDCA). Products derived from precision fermentation must achieve the status of “generally recognized as safe” (GRAS), which allows their use in food without the need for additional approvals, if sufficient scientific evidence supports their safety. In the European Union, the European Food Safety Authority (EFSA) evaluates precision-fermented proteins as “novel foods” under the Novel Foods Regulation (EU) 2015/2283, given their lack of prior consumption history before May 1997. Similarly, in Australia and New Zealand, Food Standards Australia New Zealand (FSANZ) regulates these proteins as novel foods under the FSANZ Food Standards Code. Other regions, including Canada, Japan, and Brazil, follow their own regulatory frameworks, which, while aligned with international standards, may vary in specific requirements and processes.

The approval process for precision-based dairy proteins generally involves several stages. One of the initial steps is achieving GRAS status in the U.S.,

often through voluntary consultation with the FDA. Companies must provide comprehensive scientific data demonstrating the product's safety based on its composition, production methods, and potential health effects. These data may include toxicological studies, allergenicity assessments, and detailed information on the genetically engineered microorganisms used in the fermentation process. In the European Union, producers of precision-fermented proteins must submit a detailed application to EFSA, which evaluates the production process and potential risks before making a recommendation to the European Commission for final approval.

A key focus for regulatory agencies is ensuring the absence of health risks such as allergenicity or toxicity. Precision fermentation for animal-identical dairy proteins can pose allergenicity risks due to the nature of the proteins produced. For example, proteins like casein and β -lactoglobulin are known dairy allergens and may trigger reactions in sensitive individuals, regardless of the production method. Additionally, fermentation processes may introduce unintended modifications, such as glycosylation, or contamination from microbial proteins, which can create new allergenic epitopes. Historical examples, like genetically modified (GM) soybeans with Brazil nut proteins causing allergic reactions, highlight the need for rigorous testing. Regulatory agencies like the FDA and EFSA mandate allergenicity assessments, including protein sequence comparisons, simulated digestion studies, and evaluation of byproducts, to ensure safety.

In addition to health and safety, regulatory agencies also assess the environmental impact and sustainability of precision fermentation processes. This includes evaluating the safety of microorganisms used in production to ensure they do not pose risks of contamination or ecological imbalance.

Labeling requirements represent another important aspect of regulatory approval. Many countries mandate transparency regarding the use of genetically modified organisms (GMOs) or novel ingredients in food products. Approved precision-based dairy proteins may require clear labeling to indicate their inclusion or specify whether they are derived from GMOs, enabling consumers to make informed choices.

Despite these well-established processes, challenges remain in achieving regulatory approval for precision-based dairy proteins. The evolving nature of precision fermentation technologies has prompted many regulatory agencies to update their guidelines to address the unique characteristics of these products. This evolution can create uncertainty for companies navigating approval pathways, potentially delaying market entry. Additionally, the lack of historical precedent for these novel proteins means regulators must rely on existing scientific data, which can be limited. Discrepancies in regulatory approaches across regions also pose challenges for companies seeking to market their products internationally, as they must navigate varying requirements and frameworks.

Nevertheless, the rigorous evaluation processes implemented by regulatory agencies are vital for ensuring the safety and quality of precision-based dairy proteins. As more scientific evidence supports the safety and efficacy of these products, regulatory frameworks are expected to become more streamlined, fostering innovation and market adoption. Precision-based dairy proteins have the potential to play a transformative role in the future of food production, offering sustainable, ethical alternatives to traditional dairy while addressing the growing global demand for environmentally friendly solutions.

2.3 Challenges in scaling and achieving commercial viability in precision fermentation for dairy protein production

Precision fermentation (PF) holds tremendous promise to revolutionize the production of dairy proteins by providing sustainable alternatives to traditional animal-based dairy. Despite its potential, this technology faces significant challenges in achieving scalability and commercial viability, particularly when transitioning from small-scale laboratory experiments to large-scale industrial production. Among the most critical issues are scalability, protein yield, productivity, and titer key metrics that determine the success of PF in dairy protein production.

A key technical challenge in scaling precision fermentation lies in transitioning from small-scale laboratory setups to full-scale industrial manufacturing, which typically ranges from 100 m³ to several million liters. While the precision fermentation (PF) sector has attracted significant investment—\$938 million in 2021 and \$382 million in 2022 [9]—many companies struggle to move beyond proof-of-concept experiments at the laboratory scale to large-scale production.

This critical phase, often called the “Valley of Death,” underscores the difficulty of replicating high protein titers (protein concentration) and productivity achieved in small-scale fermentations (1–5 L) in commercial fermenters of 100,000 L or more. Successfully overcoming this challenge is crucial to ensuring that PF technologies are both scalable and cost-effective.

For food applications like dairy proteins, which generally have lower market prices, achieving high protein yields and productivities is essential to maintaining competitive production costs. However, for high-value dairy proteins such as lactoferrin, the economic thresholds are more forgiving due to the higher market value of these products.

One of the specific challenges during scaling is oxygen transfer [10], particularly in fungi-based systems commonly used for dairy protein production. At small scales, oxygen levels in fermentation tanks can be more easily controlled, allowing microorganisms such as *Trichoderma reesei* or *Aspergillus oryzae* to thrive. However, as fermentation processes scale up, limitations in oxygen transfer and reactor design become more pronounced, leading to reduced growth and productivity of the microorganisms. Without adequate infrastructure to address these scaling issues, protein yields and productivities may decrease significantly, undermining the economic viability of the entire process.

Despite these challenges, precision fermentation continues to drive innovation in the food industry. Companies are tackling scalability issues through advancements in reactor design, optimized fermentation strategies, and genetic engineering to address the “Valley of Death.” While obstacles such as protein yield, production efficiency, and recovery persist, the potential of PF to replace traditional animal-based dairy products with sustainable alternatives remains a compelling incentive for ongoing investment.

With continuous technological advancements, precision fermentation is emerging as a scalable and cost-effective solution for producing dairy proteins, promising a transformative shift in global food production and consumption. **Table 1** highlights over 35 companies actively engaged in producing dairy and human breast milk proteins using PF, with the majority founded within the past five years.

Company	Year	Country	Dairy Protein	Host	Company	Year	Country	Dairy Protein	Host
Perfect day	2014	USA	BLG, Casein	Fungi	Fermify	2021	Austria	Casein	Bacteria
New culture	2018	USA	Casein	Bacteria	Bon Vivant	2021	France	Casein	n.d.
Change foods	2019	Australia/USA	Casein	Bacteria	Nutropy	2021	France	Casein	Yeast
Changjin Biotechnology	2019	China	Casein/BLG	n.d.	Phyx44 Labs	2021	India	Casein, BLG	n.d.
Formo	2019	Germany	Casein	Yeast	De Novo Foodlabs	2021	South Africa	Lactoferrin	Yeast
Remilk	2019	Israel	Lactoferrin, BLG	Yeast	Real Deal Milk	2021	Spain	Casein	Yeast
Harmony baby nutrition	2019	USA	Human milk protein	n.d.	Maya Milk	2021	Turkey	BLG	Yeast
Helainia	2019	USA	Lactoferrin	Yeast	Future Cow Tech	2022	Brazil	Casein, BLG	n.d.
Turtletree	2019	USA/Singapore	Lactoferrin	n.d.	Updairy	2022	Brazil	Casein, BLG	Fungi
All G Foods	2020	Australia	Lactoferrin	n.d.	DairyX	2022	Israel	Casein	Yeast
Those Vegan cowboys	2020	Belgium	Casein	n.d.	PFX Biotech	2022	Portugal/ Belgium	Lactoferrin, Osteopontin	Yeast
21st bio	2020	Denmark	BLG	Fungi	Ark Bio	2022	Brazil	Lactoferrin	n.d.
Standing ovation	2020	France	Casein	n.d.	Harvest Moon Foods	2023	Canada	Casein	n.d.
Zero cow factory	2020	India	Casein	Yeast	Danone/DMC	2023	France	n.d.	n.d.
Imagindairy	2020	Israel	Casein, BLG	Fungi	FrieslandCampina/ Triplebar	2023	Netherlands	Lactoferrin	n.d.
Daisy lab	2020	New Zealand	Casein	Yeast	Vivici	2023	Netherlands	BLG	Yeast
Better dairy	2020	UK	Casein	Yeast	Upli Bio	2024	UK	Human milk protein	n.d.
Eden brew	2021	Australia	Casein	Yeast					

n.d. Data are not disclosed, BLG: β -lactoglobulin.

Table 1.
Precision fermentation companies in dairy and human milk protein production.

2.4 Case study 1: Perfect day - Producing whey protein and casein through precision fermentation

Perfect Day was founded in 2014 by Ryan Pandya and Perumal Gandhi in California USA with a vision to transform the food industry. Motivated by their personal experiences with the limitations of plant-based dairy alternatives, the founders aimed to create a solution that delivered the taste, texture, and functionality of traditional dairy without involving animals. Their groundbreaking approach focused on precision fermentation, a process that uses microorganisms to produce animal-identical proteins.

The journey began with the ambitious goal of producing casein and whey, the primary proteins in milk, using genetically modified microbes. By inserting dairy protein-producing genes into microorganisms like filamentous fungi, Perfect Day pioneered the fermentation of proteins that are molecularly identical to those found in cow's milk. After years of research and development, the company achieved a major breakthrough: the successful production of β -lactoglobulin (BLG), a key whey protein. This milestone proved the feasibility of creating animal-identical dairy proteins without the need for cows.

Scaling this technology from laboratory experiments to commercial production was a significant challenge. Perfect Day partnered with fermentation facilities to expand production capacity and refine processes for large-scale manufacturing. By addressing bottlenecks in fermentation efficiency and protein recovery, the company made substantial improvements in yield and cost-effectiveness.

In 2020, Perfect Day brought its animal-free dairy proteins to market [11], forming the basis for a variety of products, such as ice cream, cream cheese, and milk. These products, developed in collaboration with food brands and retailers, garnered widespread praise for their ability to replicate the taste, texture, and functionality of traditional dairy, providing consumers with a sustainable and ethical alternative. In past two years, Unilever and Nestlé began incorporating Perfect Day's whey protein into their product lines [12, 13], marking a significant milestone in the adoption of precision fermentation technology.

2.5 Case study 2: TurtleTree - Producing lactoferrin through precision fermentation

TurtleTree was founded in 2019 by Fengru Lin and Max Rye in Singapore, with the goal of transforming the dairy industry by producing high-value bioactive ingredients through innovative technologies. Motivated by the potential of cellular agriculture to overcome the limitations of traditional dairy production, the founders focused on lactoferrin, a multifunctional protein in milk known for its significant nutritional and health benefits.

The company's approach centers on precision fermentation, utilizing genetically engineered microorganisms to produce animal-identical proteins. TurtleTree specifically targeted lactoferrin for its critical role in infant nutrition, immune health, and other therapeutic applications. By introducing lactoferrin-producing genes into microorganisms, TurtleTree developed a fermentation process capable of producing the protein with the same molecular structure as natural lactoferrin.

In November 2023, TurtleTree achieved a groundbreaking milestone by obtaining the world's first Self-GRAS (Generally Recognized as Safe) status for animal-free lactoferrin, officially approved for commercialization in the U.S. [14]. This approval

enabled TurtleTree to bring its precision-fermented lactoferrin to the market, marking a major advancement in the production of functional dairy proteins.

Currently retailing between \$750 and \$1500 per kilogram [15], the demand for lactoferrin heavily outweighs its supply, with an increasing number of consumers seeking its benefits. Since it takes at least 3000 liters of milk to extract just 1 kilogram of purified lactoferrin [16], the supply remains limited, and much of it is reserved for essential products like infant formula, leaving little for other applications. TurtleTree's approach to precision fermentation offers a sustainable solution to meet this growing demand.

TurtleTree's success with lactoferrin showcases the transformative power of precision fermentation to reshape the food and health industries.

2.6 Emerging alternatives to precision fermentation: Cellular agriculture and molecular farming for animal-identical dairy and human milk proteins

In addition to precision fermentation, two new approaches, namely cellular agriculture and molecular farming, are emerging as promising alternatives for producing animal-identical dairy proteins and human breast milk proteins. These innovative technologies provide alternative pathways for creating sustainable and ethical protein sources, each with its own unique advantages and challenges.

2.6.1 Cellular agriculture

Cellular agriculture offers an alternative to precision fermentation by cultivating animal cells directly to produce milk proteins. Unlike precision fermentation, which uses microorganisms to produce proteins, cellular agriculture involves growing mammalian cells, such as those from cows or humans, in bioreactors. These cells are nurtured in controlled environments and induced to produce milk, closely replicating the natural secretion processes that occur in living animals [17].

A key advantage of cellular agriculture is its potential to produce authentic, biologically relevant animal proteins, including those found in human milk. By using human mammary cells, cellular agriculture can directly generate proteins present in human breast milk, such as lactoferrin, antibodies, and also oligosaccharides, thereby addressing the nutritional and immune benefits of natural breast milk. However, scaling this process remains a challenge, as it requires optimizing cell culture and bioreactor conditions to achieve high yields in a cost-effective manner.

2.6.2 Molecular farming

Molecular farming is another alternative method that uses genetically engineered plants to produce animal-identical proteins [18]. This approach involves inserting genes that encode specific milk proteins (such as casein, whey, or lactoferrin) into the DNA of plants like alfalfa, carrots, rice, or tobacco. These modified plant cells then express the desired proteins in their tissues, which can be harvested and purified for commercial use.

Molecular farming offers scalability and lower production costs, as plants are inexpensive to grow and require fewer resources than large fermentation tanks. This method is particularly attractive for producing large quantities of proteins such as casein, which are essential for animal-free dairy products. However, challenges, such as protein purification and the development of stable modified plant cells with high yields, must be overcome for it to become a widely adopted solution.

Company	Founding year	Country	Dairy protein	Company	Founding year	Country	Dairy protein
<i>Molecular Farming</i>				<i>Cultivated</i>			
Alpine bio	2016	USA	Casein	Wilk	2018	Israel	Human milk
Mozza foods	2018	USA	Casein	BiomilQ	2019	USA	Human milk
Fantastic farms	2019	Israel	Casein	Opalia	2020	Canada	Dairy
Miruku	2020	New Zealand	Casein	Brown Foods	2021	India	Not disclosed
Ergo bio	2020	USA/ARG	Casein	Senara GmbH	2022	Germany	Dairy
NewMoo	2021	Israel	Casein	Numi	2023	France	Human milk
Veloz	2021	Mexico	Casein	Hulk Bio	2024	UK	Human milk
Kyomei	2021	UK	Casein				
Aspyre Foods	2022	USA	Casein				
Nobell Foods	2022	USA	Casein				
Kinish	2023	Japan	Casein				
Finally Foods	2024	Israel	Casein				

n.d. Data are not disclosed.

Table 2.

List of molecular farming and cultivated milk companies.

While precision fermentation remains the dominant method for producing animal-identical dairy proteins, both cellular agriculture and molecular farming offer exciting alternatives. Cellular agriculture can produce complex human milk proteins directly, potentially offering more biologically accurate and functionally relevant products. Molecular farming, on the other hand, presents an efficient, plant-based alternative for producing high-volume dairy proteins at a lower cost. Both approaches are evolving rapidly and may provide scalable, sustainable solutions to meet the growing demand for animal-identical proteins in the future. **Table 2** shows the companies developing these technologies.

3. Conclusions

The future of dairy protein production through precision fermentation holds transformative potential for the food industry. As technologies continue to advance, we can expect a broader range of dairy ingredients ranging from proteins to fats to be produced using fermentation. This will not only help meet the growing consumer

demand for plant-based alternatives but will also address global sustainability challenges.

The successful commercialization of precision-fermented dairy proteins has already proven that large-scale production is achievable, and the industry is now focused on expanding this to include dairy fats. With continued technological innovations and regulatory approvals, precision fermentation is set to reshape the dairy landscape, offering sustainable, ethical, and high-quality alternatives to traditional dairy products.

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Conflict of interest


The authors declare no conflict of interest.

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Section 2

Chemical Composition and
Techno-Functional Properties
of Milk and Dairy Products

Milk Proteins: Foaming and Emulsifying Properties in Dairy Processing

Roua Lajnaf, Hamadi Attia and Mohamed Ali Ayadi

Abstract

Milk proteins, well-known for their nutritional properties, have also interesting techno-functional properties including foaming and emulsifying properties. Indeed, they play a crucial role in the creation and stabilization of foams and emulsions, making them one of the most essential components in manufacturing dairy products. First, major proteins in milk (α_s -, β - and κ -caseins as well as α -lactalbumin and β -lactoglobulin) are presented and their biological and physicochemical characteristics are highlighted. Furthermore, this chapter explores the recent researches about foaming and emulsifying properties of milk proteins, focusing on their molecular interactions, mechanisms of stabilization, and the impact of processing conditions such as pH value and thermal treatments. Key factors influencing foam and emulsion stability, such as the impact of emerging technologies in dairy industry including high pressure homogenization, high pressure jet processing, ultrasound, and sonication, are also discussed. Understanding these properties is essential for optimizing product formulation and ensuring the quality and texture of dairy-based foods.

Keywords: foam, emulsion, caseins, whey proteins, dairy process

1. Introduction

Techno-functional properties are defined as the expression of physical or physicochemical characteristics in terms of the sensory quality of food, particularly in food systems that have undergone stabilization or technological processing. In the food industry including dairy one, proteins are the primary ingredients used for their techno-functional properties, occupying a prominent position due to their high nutritional and techno-functional value [1]. The techno-functional properties of proteins result from two main factors:

- *Physicochemical and structural characteristics* of the protein, including molecular weight (MW), amino acid composition and distribution, and surface charge.
- *External factors* such as pH, temperature, ionic strength, and the emerging food processing that can be applied to the food system.

Techno-functional properties of proteins are commonly classified into three main categories, as illustrated in **Figure 1**.

- *Hydration properties*, which depend on protein–water interactions. These include water absorption and retention, wettability, adhesion, swelling, solubility, dispersibility, and viscosity.
- *Structuring properties*, which depend on protein–protein interactions, encompassing precipitation, coagulation, and gelation.
- *Surface properties*, which involve the interaction of proteins with liquid (emulsifying properties) or gaseous phases (foaming properties). Multiphase food systems typically include two types of interfaces: the oil/water interface, found in emulsions, and the air/water interface, present in foams.

The formation of emulsions and foams is based on the same principle: the creation of an interface followed by the migration and spreading of surface-active agents. Indeed, contact between two immiscible phases (e.g., water/oil) is thermodynamically unfavorable. As a result, the interfacial energy—or interfacial tension—is high and leads to the destabilization of the system. The ability of proteins to adsorb at the interface is determined by their capacity to reduce interfacial tension. This capacity is associated with the amphiphilic nature of the protein, that is, the simultaneous presence of hydrophilic and hydrophobic regions on the molecular surface. During protein adsorption at the interface, proteins orient themselves so that their hydrophilic and hydrophobic regions interact with the aqueous and hydrophobic phases (oil or air), respectively, to minimize interfacial tension. The creation of an interface (air/water or oil/water) is a purely mechanical phenomenon, requiring an input of energy into the system—most often in the form of mechanical energy. For instance, in the food industry, homogenizers are used to produce emulsions, while whipping devices are employed to create foams [2].

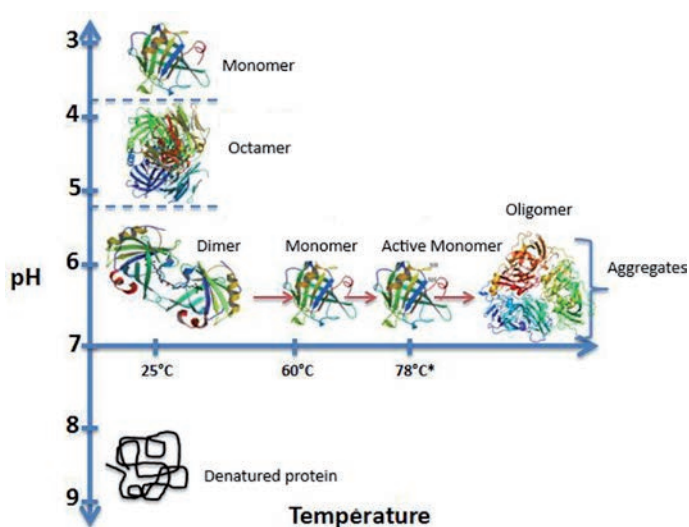


Figure 1. Influence of extrinsic factors on the structure of β -lactoglobulin (pH and temperature).

2. Characteristics of milk proteins

Milk proteins represent a significant nutritional contribution as they are a source of essential amino acids. They also play a crucial role in the techno-functional properties important for the preservation and processing of milk into derivative products [3].

Milk proteins are classified into two fractions based on their solubility: caseins (insoluble in acidic conditions) representing colloidal fraction of milk and whey proteins (also known as soluble proteins) representing the soluble fraction. Caseins precipitate at their isoelectric point, which is around pH 4.6 for cow's milk, whereas whey proteins remain soluble at this pH range. They also can be obtained using different methods such as rennet coagulation or ultracentrifugation at a speed of 100,000 g [4, 5].

2.1 Caseins

Caseins are phosphoproteins and represent the most abundant protein fraction in milk accounting for 80% (w/w) in cow's milk. Caseins are predominantly found in milk in the form of large colloidal structures formed through supramolecular assembly. These structures, known as casein micelles, typically have an average size ranging between 120 and 150 nm in cow's milk but can vary according to mammalian producing species of milk. Within the mammary secretory cells, caseins play a key role by transporting substantial quantities of calcium phosphate, which is otherwise poorly soluble. This function ensures the delivery of essential nutrients to infants through milk [6, 7]. Caseins consist of four different proteins: alpha-S1, alpha-S2, beta, and kappa (α S1, α S2, β , and κ) in different proportions 36, 38, 10, and 13% for β -, α S1-, α S2-, and κ -caseins, respectively [8]. The α - and β -caseins are referred to as calcium-sensitive caseins, due to their precipitation at calcium concentrations of approximately 30 mM, whereas κ -casein remains in solution under these conditions. The κ -casein is known by its key role in the enzymatic coagulation process of milk [9].

2.1.1 α -Casein

Alpha-s casein, which includes α _{S1}- and α _{S2}-caseins, is the most abundant protein in cow's milk, with a concentration estimated at 12.8 ± 2.3 g/L [10]. First, the α _{S1}-casein in cow's milk is present at a concentration of approximately 9.5 g/L according to the studies of Omar et al. [10] using capillary electrophoreses, representing about 38% of total caseins. This consists of 199 amino acid residues and has an estimated MW of 22.9 kDa. Its isoelectric point is estimated at pH 4.26. A second isoform (variant A), composed of 207 amino acids, has been identified through chromatography, resulting from the deletion of eight residues due to alternative mRNA splicing during transcription [11]. Bovine α _{S1}-casein is distinguished from other caseins in milk by the absence of cysteine residues contains eight serine residues in phosphorylated form. Due to the high number of proline residues (8.5% in bovine α _{S1}-casein), this protein lacks ordered secondary structures such as α -helices or β -sheets [12].

Meanwhile, the α _{S2}-casein is the most hydrophilic of the caseins, with 11 phosphorylated serine residues and two cysteine residues (positions 36 and 40) that form intramolecular disulfide bonds. This casein is partly found in dimeric form, with two polypeptide chains linked by disulfide bridges. Its secondary structure contains approximately 32% α -helices and 30% β -sheets, suggesting a more ordered and structured conformation compared to α _{S1}-casein [11].

2.1.2 β -Casein

The β -casein is one of major milk proteins with a concentration of 11.7 ± 0.9 g/L, representing a significant proportion of the total casein content [10]. It β -casein is composed of a single polypeptide chain consisting of 209 amino acids and has a MW of approximately 24 kDa (**Table 1**). It accounts for about 33–45% of the total casein content in cow's milk, with typical concentrations ranging from 9.0 to 11.0 g/L [13]. It is the second most prevalent casein type in bovine milk. Due to its abundance and unique structural features, β -casein has drawn significant interest from researchers. Like other caseins, it is classified as an intrinsically disordered protein due to its high proline content (16.7%) leading to a lack of a well-defined tertiary structure and exhibits its notable amphiphilic properties. This protein interacts with calcium phosphate to form complex aggregates known as casein micelles. One of the distinguishing features of β -casein is its high content of phosphorylated serine and proline residues, which contributes to its strong hydrophobicity and excellent calcium-binding capacity [14]. Among all caseins, β -casein is the most hydrophobic and displays a high amphipathic character. Its C-terminal region (residues 136–209) is rich in nonpolar amino acids, while the N-terminal segment (residues 1–40) is hydrophilic and contains phosphorylated residues that confer additional negative charges to the molecule. This protein lacks disulfide bonds, which contributes to its notable resistance to heat treatments.

β -Casein exhibits considerable genetic diversity, with 13 distinct variants identified so far, including A1, A2, A3, B, C, D, E, F, G, H1, H2, I, and J. Among these, the A1 and A2 forms are the most widespread. Indeed, milk that exclusively contains the A2 variant is referred to as “A2 milk,” whereas milk containing only the A1 variant is known as “A1 milk.” A2 β -casein is considered the ancestral form found in early domesticated cows, while the A1 variant has become more prevalent due to a naturally occurring genetic mutation and subsequent selective breeding [18]. Its unique structural features—such as intrinsic disorder, high hydrophobicity, relatively low molecular weight, and absence of disulfide bonds—make it a key contributor to the functional properties of dairy protein ingredients, especially in applications where stabilization is required [19].

2.1.3 κ -Casein

The κ -casein is the key milk protein involved in the enzymatic coagulation of milk by rennet. In cow's milk, it is present at a concentration of 4.4 ± 0.3 g/L, accounting

Protein	Amino acid residues	Molecular weight (kDa)	Concentration (g/L) and relative amounts (% of casein/whey fraction)	Isoelectric point	References
α_{S1} -casein	199	22.975	10.0–15.0 (38.0%)	4.26	[8, 13–15]
α_{S2} -casein	207	24.348	3.0–4.0 (10.0%)	4.78	
β -Casein	209	23.583	9.0–11.0 (36.0%)	4.49	
κ -Casein	169	18.974	3.0–4.0 (13%)	3.79	
β -Lactoglobulin	123	18.281	3.0–4.0 (55%)	4.65	[13, 15–17]
α -Lactalbumin	162	14.186	10–1.5 (25%)	4.66	

Table 1. Physicochemical characteristics of caseins and whey proteins in cow's milk.

for approximately 13% of the total casein fraction [10]. This protein consists of 169 amino acid residues and has a molecular mass of 18,974 Da and an isoelectric point of 3.97 [20]. Structurally, κ -casein displays amphipathic properties similar to those of β -casein, characterized by a hydrophilic C-terminal region enriched with glycosylated residues and a hydrophobic N-terminal domain. Unlike other caseins, κ -casein has a limited calcium-binding capacity due to the presence of only one phosphorylation site located at position 149. It is well established that partial hydrolysis of bovine κ -casein by chymosin occurs at the peptide bond between phenylalanine 105 and methionine 106. This cleavage results in the release of a highly hydrophilic fragment known as the caseinomacropptide (CMP), which comprises 64 amino acids with a molecular weight of approximately 6.7 kDa, and the formation of para- κ -casein, an insoluble, hydrophobic peptide that plays a critical role in micelle destabilization and clot formation.

2.2 Whey proteins

Whey proteins constitute the soluble portion of milk, where casein micelles are dispersed. These proteins make up the second most significant group in bovine milk, accounting for approximately 20% of its total protein content [21]. These proteins represent the by-product fraction after cheese making regardless of using processing method. Whey is particularly rich in well-organized globular proteins, among which β -lactoglobulin is the most prevalent. This protein represents more than the half of all whey proteins and notably lacks a counterpart in human milk [22].

2.2.1 β -Lactoglobulin

The β -lactoglobulin is a globular milk protein found in the milk of most mammals, with the notable exception of camelids, primates, and rodents. It plays a biological role in transporting fatty acids, retinol, and fat-soluble vitamins (A and D) during digestion [23].

In cow's milk, β -lactoglobulin is the predominant protein in the soluble fraction, with a concentration ranging between 2 and 4 g/L (**Table 1**), accounting for approximately 55% of the whey proteins. However, this protein is absent in camel and human milk whey, as reported by several studies [16, 24].

The primary structure of bovine β -lactoglobulin consists of 162 amino acids, with a molecular weight of 18.281 kDa and an isoelectric point (pI) of 5.2. Its secondary structure is composed of approximately 10% α -helices, 45% β -sheets, and 15–20% β -turns. The protein contains two intramolecular disulfide bridges (Cys66-Cys160 and Cys106-Cys119) and one free thiol group (Cys121) [23]. β -Lactoglobulin displays various quaternary structural forms, with several stages of oligomerization depending on environmental conditions such as pH, temperature, and ionic strength. These factors primarily influence the equilibrium between monomeric, dimeric, and octameric forms:

- *pH*: At pH 3.5, high electrostatic repulsion leads to dissociation of dimers into monomers. At pH values above 8, β -lactoglobulin undergoes an irreversible conformational change, resulting in protein monomer dissociation and aggregation, particularly beyond pH 9 [25].
- *Temperature*: β -lactoglobulin experiences significant structural changes upon heat treatment. However, the thermal response is not solely dependent on

temperature but also influenced by pH and ionic strength (**Figure 2**). For instance, under neutral pH and low ionic strength, dimeric β -lactoglobulin dissociates into native monomers at 60°C. Above 78°C—the denaturation threshold—these monomers unfold and aggregate into oligomers *via* intermolecular disulfide bond exchange [26]. At room temperature and near-neutral pH (such as milk pH ~6.7), β -lactoglobulin predominantly exists in a dimeric state (~36.7 kDa). As pH decreases, these dimers can polymerize into octamers.

2.2.2 α -Lactalbumin

The α -lactalbumin is a small protein composed of 123 amino acids (**Table 1**), known for its richness in essential amino acids and its crucial role in lactose biosynthesis, in conjunction with lactose synthase and UDP-galactosyl transferase [27].

The α -lactalbumin is a metalloprotein that binds one Ca^{2+} ion per mole of protein, a divalent cation that plays an important role in stabilizing its three-dimensional structure. Calcium binding occurs through the acidic side chains of aspartic acid residues located at positions 82, 87, and 88. A secondary calcium-binding site, typically occupied by zinc, exists but with an affinity 10^5 times lower than that of calcium. It contains four disulfide bridges (Cys6-Cys120, Cys28-Cys111, Cys61-Cys77, and Cys73-Cys91), and no free thiol groups. This configuration provides the protein with higher resistance to heat-induced aggregation, although its denaturation temperature is relatively low (~64°C) [28]. The tertiary structure of α -lactalbumin consists of two distinct domains:

- A β -domain, composed mainly of β -sheets, rich in acidic residues (10 Asp), forming the Ca^{2+} binding site, with an isoelectric point (pI) of 3.37.
- An α -domain, formed by four α -helices creating a hydrophobic core, is basic in nature due to the presence of 9 lysine residues, with a pI of 9.6.

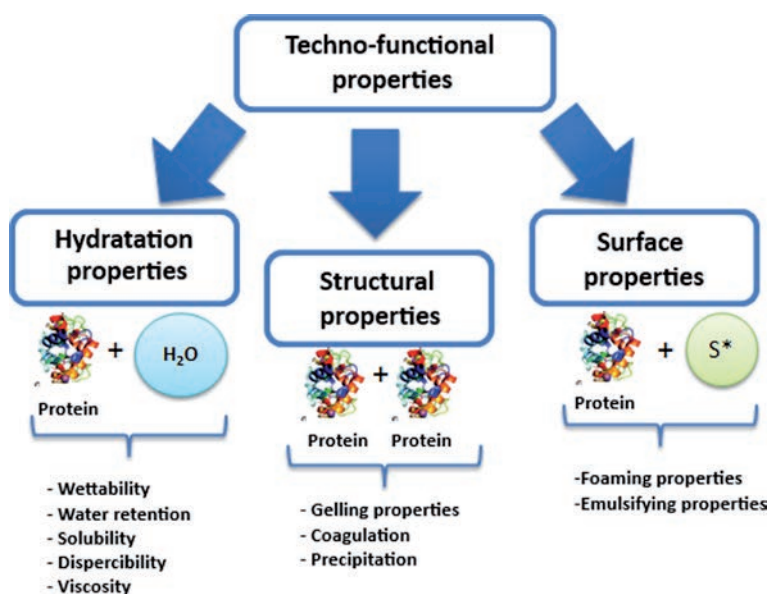


Figure 2. Different classes of techno-functional properties of proteins S*: Surface (oil or air).

The α -lactalbumin is found in a monomeric form at a concentration of approximately 1.2 g/L in cow's milk. However, at acidic pH values (<5), α -lactalbumin undergoes significant conformational changes, losing its calcium ion and adopting a compact, fluctuating tertiary structure referred to as the molten globule state [29, 30].

This calcium-free form, known as apo- α -lactalbumin, is less thermally stable. In contrast, the calcium-bound form, holo- α -lactalbumin, plays a stabilizing role under reducing conditions. Native α - α -lactalbumin denatures at around 64°C but can refold upon cooling, regaining 80–90% of its native structure. However, exposure to 95°C for 15 minutes significantly reduces refolding efficiency to around 40%. Dimeric and tetrameric forms of α - α -lactalbumin appear after heat treatment. Above 90°C, α - α -lactalbumin undergoes irreversible denaturation through the cleavage of intramolecular disulfide bridges, leading to the formation of dimers and tetramers [31]. At 95°C and beyond, these changes result in the formation of large protein aggregates (**Figure 2**).

2.2.3 Minor whey proteins

In addition to the major whey proteins, α -lactalbumin and β -lactoglobulin, whey also contains several other proteins that are considered minor components but possess important biological functions. Among these, bovine serum albumin (BSA) and lactoferrin stand out due to their distinctive structural and functional characteristics. BSA is a whey protein with a relatively high molecular weight. It consists of 583 amino acids and has a molecular mass of approximately 66.4 kDa. Its primary sequence was determined by Hirayama et al. [32]. BSA contains 17 intramolecular disulfide bonds and a single free thiol group, contributing to its structural stability and its ability to bind a wide range of ligands. Lactoferrin is another minor whey protein (691 amino acid residues; 80 kDa), known for its multifunctional roles, particularly its antimicrobial activity and high affinity for iron. Despite their lower abundance compared to the major whey proteins, these minor proteins significantly enhance the nutritional and bioactive profile of milk [33].

3. Milk proteins: Processing and technological aspects of foams and emulsions

The structural configuration and physical characteristics of milk proteins contribute to their wide application in various techno-functional roles, including water retention, emulsification, and foaming. These functionalities can be exploited either in their natural form or after undergoing specific treatments such as enzymatic, physical, or chemical modifications. Overall, for proteins to exhibit effective foaming or emulsifying behavior, they must possess solubility, amphiphilic nature, and surface activity, along with the capacity to readily adjust their conformation at newly formed interfaces [1, 34].

3.1 Foaming properties of milk proteins

According to Dickinson et al. [35], foam is defined as a dispersion of gas bubbles within a continuous phase, which may be liquid, semisolid, or solid. Foaming refers to the increase in volume resulting in the formation of a foam structure. A typical foam contains approximately 1000 bubbles per milliliter. It is considered a colloidal system in which air bubbles are separated by thin liquid films and stabilized

by a protein matrix at the air–water interface. This interfacial protein layer plays a crucial role in maintaining the stability and structure of the foam [36]. Foaming is a widely used operation in food manufacturing, particularly in aerated food products including cappuccino, whipped cream, ice cream, mousses, toppings, meringues, sponge cakes, marshmallows, and aerated chocolate. These foamed food products are typically classified according to the food type, the method used to create the foam, and the characteristics of the final foam structure [37, 38]. In the case of milk-based foams where skimmed milk and/or milk proteins were used as raw materials, they are considered colloidal systems where air bubbles are supported by a matrix formed from milk constituents [39].

For milk foams, proteins in milk serve as the main surface-active agents responsible for foam creation and stabilization. Their foaming capacity stems from their ability to: (1) adsorb at the air-water interface leading to the reduction of the surface tension; (2) unfold at the interface and orient their hydrophilic and hydrophobic groups toward the aqueous and air phases, respectively; and finally, (3) form cohesive films through interactions involving partially denatured protein molecules [40]. Research into milk foaming dates back to the early twentieth century, when efforts primarily aimed to minimize foam formation during milk processing. While this remains a concern, current research tends to focus more on understanding and enhancing the foaming behavior of individual milk proteins [30, 41–43]. Nonetheless, studies examining the foaming dynamics of whole milk, which includes a complex mixture of proteins, remain limited.

3.1.1 Effect of pH on foaming properties

Milk proteins molecules (caseins and whey proteins) change their conformation and surface activity depending on pH value. Therefore, foaming properties also change according to this physicochemical parameter [34].

For instance, Zhang et al. [44], who investigated the foaming characteristics of milk protein mixtures—composed of whey protein isolate and milk protein concentrate—reported notable findings regarding their functional behavior across a pH range of 3.0–9.0. Their study assessed foaming capacity, foam stability, and foam morphology, alongside evaluations of physicochemical, structural, and interfacial properties, supported by Pearson correlation analysis to better understand foam performance. According to the results, milk proteins mixture exhibited excellent FC (113.0–114.3%) and FS (90.7–93.0%) particularly in the pH range of 6.0–9.0, producing foams with smaller and more uniformly distributed bubbles. These authors highlight the potential of milk proteins in a mixture as functional components in the formulation of aerated food products under varying pH conditions. Meanwhile, Zhao et al. [45] who explored the impact of alkaline pH-shifting combined with ultrasound treatment on various milk proteins—including milk protein concentrate, micellar casein concentrate, and whey protein isolate—reported significant improvements in their physicochemical characteristics, solubility, and foaming performance. Their findings showed that solubility markedly increased with intensified ultrasound-assisted pH-shifting. Moreover, the treatment substantially reduced particle size in both milk protein and micellar casein concentrates and decreased protein turbidity. The foaming capacity of all three protein types improved notably under alkaline conditions, with the most pronounced enhancement observed at pH 11. At this pH level, the proteins also exhibited maximum surface hydrophobicity. The enhanced foaming ability was attributed to a combination of factors, including reduced particle size, altered zeta potential,

changes in protein structure, increased solubility, and higher surface hydrophobicity. Overall, Zhao et al. concluded that ultrasound-assisted pH-shifting, particularly under alkaline conditions, is an effective strategy for enhancing the functional properties of milk proteins—especially micellar casein concentrate—highlighting its potential for applications in aerated food products within the food industry.

For individual proteins, foaming proteins depended on pH value. First, individual whey proteins such as β -lactoglobulin foams more at a pH close to 4.5, pH close to the pI of this protein (pI = 5.2). The schematic modeling of whey protein adsorption layers is not represented by the β -lactoglobulin, as the major protein as observed for sodium caseinates which was represented by β -casein. Thus, Marinova et al. [3] represented the adsorbed layer at the air water interface by globular proteins including β -lactoglobulin and α -lactalbumin that adsorb almost intact at the interface. This average of whey proteins is negatively charged at neutral pH, and electrostatic repulsions disable the formation of a dense protein adsorption layer. Meanwhile, in acidic conditions, the adsorbed molecules that are not charged interact leading to dense layer.

3.1.2 Effect of temperature on foaming properties

Temperature plays a critical role in preserving foods including dairy products. Meanwhile, thermal treatments are directly implied in altering the conformation of milk proteins and influencing their partitioning between the whey and colloidal phases of milk [40]. As a result, it impacts not only the molecular structure of these proteins but also their surface-active properties, which in turn affect their ability to form and stabilize foams [46].

First, Oetjen et al. [47] who studied the foaming behavior of four milk types—ultra-high-temperature (UHT) processed with 1.5% and 3.5% and pasteurized milk with 1.5% and 3.5% of fat content reported significant differences in foamability and foam stability across a temperature range from 5°C to 60°C. Their results evidenced the effect of both parameters in foam performance. Thus, at 40°C, foam stability varied noticeably among the different milk samples, a variation attributed to the distinct initial bubble-size distributions observed in each case. Additionally, they assessed the evolution of bubble size over time at both room temperature and 40°C, providing insight into how thermal conditions influence foam structure and persistence. Similarly, whey proteins in sweet cheese whey improve their foaming parameters upon heating process at a temperature of 85°C during 750 s according to the studies of Tosi et al. [48]. This behavior was explained by the change in conformation of both of the major proteins in whey α -lactalbumin and β -lactoglobulin by the exposure of the buried hydrophobic parts of these globular proteins leading to improve their tensioactive properties. Meanwhile, Lajnaf et al. [49] who compared foaming properties of camel and bovine milk after thermal treatments at different heating temperatures ranging from 70°C to 100°C reported that foaming properties (foaming capacity and stability indexes) increased with increasing the temperature up to 90°C. This temperature corresponds to the onset of protein aggregation, as evidenced by a reduction in surface hydrophobicity and net negative charge, along with the disappearance of chromatographic peaks associated with β -lactoglobulin and α -lactalbumin in cow's milk. Finally, Borcherding et al. studied the effects of foaming temperature and heat treatment on skimmed milk and found that both factors significantly influenced foam structure and stability. Higher temperatures improved foam density and stability, while thermal processing altered bubble size and decreased foam density. Unheated milk produced the most

stable foam, and microscopy revealed that foam interfaces mainly involved proteins rather than casein micelles. Indeed, the air-water interface in bovine milk was found to be maintained by the β -lactoglobulin, the α -lactalbumin, as well as the β -casein [50].

3.1.3 Effect of novel processing technologies on foaming properties

Emerging processing technologies such as ultrasonication and high-pressure processing have shown great potential in modifying the structural and functional properties of milk proteins. These treatments can enhance protein solubility, reduce particle size, and alter surface characteristics, all of which influence the foaming behavior of dairy systems. By improving interfacial activity and foam stability, such innovative approaches offer promising applications for the development of high-quality aerated dairy products and improved processing performance in the dairy industry. Maklin et al. [51] studied the influence of ultrasonication duration on milk foam characteristics and reported that varying the sonication time (from 1 to 10 minutes) significantly impacted fat globule size, viscosity, and foaming performance. These authors found that reducing the size of native fat globules generally increased viscosity. The NanoFoamer emerged as the most effective method in balancing foamability and stability. However, foaming efficiency declined after 7 minutes of sonication, suggesting that excessive sonication may negatively affect foam quality. Compared to untreated milk, ultrasonicated samples exhibited improved foam stability and initial bubble structure due to smaller fat globules. Nevertheless, prolonged sonication led to foam destabilization, likely due to disrupted protein interactions and altered fat globule membrane composition. Finally, this work highlights the importance of optimizing ultrasonication time to enhance milk foam quality and support advancements in dairy product development and consumer satisfaction.

On the other hand, Hettiarachchi et al. [52] investigated the impact of high-pressure jet processing on the foaming properties of skim milk and reported that pressures equal to or above 300 MPa significantly enhanced foam expansion. They also observed improved foam volume stability, particularly at 400 and 500 MPa, where structural alterations such as the formation of large protein aggregates were noted. These changes, attributed to modifications in casein micelle structures, were found to play a key role in enhancing the interfacial behavior of milk proteins during foaming. Their findings suggest that this novel processing can effectively improve foaming functionality, offering promising potential for the development of innovative milk-based products.

3.2 Emulsifying properties of milk proteins

An emulsion is a colloidal system formed by the dispersion of one immiscible liquid into another, typically oil in water, through a homogenization process in the presence of one or more emulsifiers. During homogenization, emulsifiers adsorb to the surface of newly formed oil droplets, reducing interfacial tension and facilitating droplet breakup. This process results in a stable dispersion of fine oil droplets within the aqueous phase. In the food industry, caseins are among the most commonly used emulsifiers due to their strong surface activity and ability to stabilize emulsions [53]. Emulsion is a structure frequently encountered in the formulation of numerous food products, including salad dressings, mayonnaise, sauces, soups, creams, and margarine. It results from the dispersion of two immiscible liquids—typically oil and water in food products—where one liquid is finely broken into droplets and distributed

throughout the other with the help of emulsifying agents. Similarly to foams, the tensioactive components called emulsifiers stabilize the oil and water mixture by reducing interfacial tension during processes like homogenization. Two types of emulsions are commonly encountered in food products oil in water emulsion and water in oil emulsion depending on the used emulsifiers, the homogenization process and the volumes of water and oil [30, 53, 54].

Among food-grade emulsifiers, proteins are the most widely used due to their superior surface activity in stabilizing emulsions, whereas phospholipids such as lecithin and polysaccharides are also used. Overall, when mechanical shear is applied during emulsification in the case of oil-in-water emulsions, oil droplets are then generated within the continuous aqueous phase. Afterwards, proteins dissolved in the water phase migrate to the oil–water interface, where they reorient themselves: hydrophilic amino acids facing the aqueous environment and hydrophobic regions aligning toward the oil. Once adsorbed, these emulsifiers unfold and form a viscoelastic film around the droplets, leading to stabilize the emulsion and preventing droplet coalescence [30, 55].

3.2.1 Effect of pH on emulsifying properties

The relationship between the structure and functionality of milk proteins has been widely explored in scientific research, particularly in terms of their tendency to aggregate and/or to precipitate as well as the various forces that govern their interactions, such as hydrophobic forces, hydrogen bonds, electrostatic attractions, and thiol-disulfide exchanges. These molecular interactions can be modified by manipulating the physicochemical characteristics of milk proteins (caseins and whey proteins). For instance, thermal treatments can be applied to partially or fully unfold the protein structure, thereby revealing hidden hydrophobic regions. Additionally, altering the pH to move closer to or further from the protein's isoelectric point can influence its surface properties and interaction behavior [26, 55].

For instance, Lam and Nickerson [55] who investigated the impact of pH levels (3.0, 5.0, and 7.0) and thermal pre-treatments (25, 55, and 85°C) on whey protein isolate, reported significant findings regarding its emulsifying performance. Indeed, these authors evaluated both of physicochemical parameters—such as surface charge, hydrophobicity, particle size, and interfacial tension—and functional emulsifying properties, including the emulsifying activity index (EAI) and emulsion stability index (ESI). The main findings in this study showed significant changes in surface charge, hydrophobicity, and aggregation affected the emulsifying activity and stability of whey proteins especially in acidic conditions in which they are characterized by higher surface hydrophobicity and lower electronegative charge. Emulsifying activity improved under conditions that minimized aggregation, while emulsion stability was enhanced at pH values distant from the isoelectric point and under lower surface hydrophobicity. Meanwhile, these authors have also studied on a single milk protein (the α -lactalbumin) in another work [30]. They found that pH value significantly influenced the emulsifying properties of the α -lactalbumin. For instance, a reduction in proteins aggregation was observed at neutral pH (pH 7) despite the high hydrophobicity. Furthermore, the created emulsions were more stable at this pH due to the higher electrostatic repulsive forces between oil droplets compared to those at pH 5 leading to the best emulsifying properties [30, 56]. Mellema and Isenbart [57], who worked on emulsifying and interfacial properties of both of whey proteins and skim milk proteins, reported that the interfacial behavior of these proteins is

significantly influenced by the studied proteins and physicochemical conditions such as pH value and thermal treatments. For instance, in the case of whey protein concentrate, acidification prior to emulsification slows protein adsorption at the oil–water interface, though the elasticity of the film remains unaffected—likely due to the eventual formation of a uniform protein layer despite slower unfolding. Conversely, acidification after emulsification leads to structural coarsening of the interfacial film, resulting in reduced rigidity. Preheating whey proteins induces protein unfolding and aggregation, yet the aggregates still exhibit interfacial activity and maintain high elastic moduli. When this thermal treatment is followed by acidification, even higher interfacial elasticity is observed. Regarding skim milk proteins, preheating has minimal influence on interfacial properties of these proteins. However, under acidic conditions, the aggregates formed tend to be less effective at stabilizing interfaces, leading to lower elasticity.

3.2.2 Effect of temperature on emulsifying properties

Thermal treatments are currently applied to food products including dairy one, in order to guarantee their safeness to consumer. This process can also affect the emulsifying properties of milk proteins and their ability to adsorb at the oil water interface as reported by many studies. First, thermal treatments of whey proteins at 85°C and at neutral pH resulted in lower emulsifying of these proteins compared to those heated at other heating temperatures (55°C and 25°C). Indeed, whey proteins were found to aggregate upon heating resulting different aggregates whose size depends on the applied temperature. Therefore, larger aggregates are expected to be obtained after heating at a higher temperature leading to lower emulsifying properties and higher emulsion stability. Thus, the emulsification of whey proteins is improved by reducing the size of aggregates. Finally, small proteins aggregates are overall characterized by a higher migration rate to the oil water interface than the large aggregates leading to higher emulsifying properties (Figure 3). Contrarily, large aggregates are characterized by lower migration rate at the oil water interface but contributed effectively to

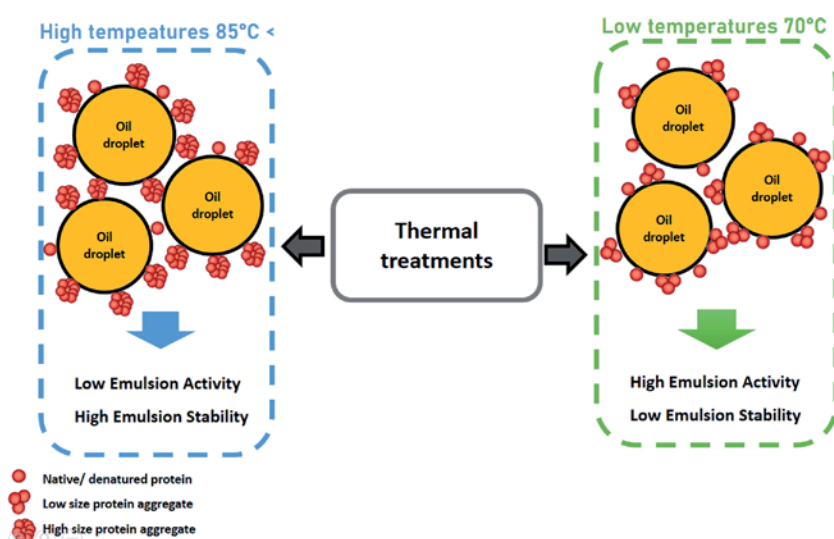


Figure 3. Effects of thermal treatments on the structural, adsorptive, and emulsifying properties of whey proteins.

the maintain of the protein film leading to higher emulsion stability [55, 58]. A recent work of Pastrana et al. [59] has reported that emulsifying rennet skimmed milk curds through a thermo-mechanical process at different temperatures ranging between 70 and 90°C resulted in the successful formation of stable emulsion gels, especially at a temperature of 85°C. Indeed, at this temperature, a well-structured gel network was obtained, while further increases led to moisture and fat loss, along with a denser protein matrix. In contrast, processing at lower temperatures (70°C) produced less stable emulsions due to incomplete gelation and oil separation. These authors highlight the critical role of thermal processing conditions in modulating the structural and functional characteristics of dairy-based emulsified systems.

3.2.3 Effect of novel processing technologies on emulsifying properties

Numerous studies have demonstrated that novel processing technologies significantly affects the emulsifying characteristics of milk proteins. Specifically, improvements in the emulsifying properties of milk proteins have been reported for whey proteins and caseins. For instance, Gao and Ma [60] observed a marked enhancement in the emulsion activity and stability indexes of whey proteins when subjected to ultrasound at varying power levels (120, 360, and 600 W) under different pH conditions. The degree of enhancement of the studied properties was influenced by both the applied ultrasonic power and the pH level of the protein solution. These authors noted that at extreme pH values ranging between pH 3 and 11, emulsion activity showed a decreasing trend with increasing power, whereas under neutral conditions, this parameter increased with higher ultrasonic input. Conversely, emulsion stability was found to increase with rising power regardless of pH value [60]. On the other hand, other processing technologies can influence the emulsifying properties of milk proteins, such as high-pressure homogenization. In this regard, Ali et al. [61] reported that high-pressure homogenization can alter the structure and emulsifying properties of β -lactoglobulin depending on the treatment intensity. Mild high pressure homogenization had little effect, while harsher conditions caused structural changes and aggregation, enhancing interfacial adsorption at low protein concentrations. However, at higher concentrations, these structural changes had minimal impact on interfacial properties or droplet size. This process as well as heat treatments produced similar effects, though differing in magnitude.

4. Conclusion

Milk proteins are characterized by interesting techno-functional properties that are currently used in manufacturing dairy products. These properties, including foaming and emulsifying one, depended on intrinsic factors such as MW, isoelectric pH, and hydrophobicity of proteins and extrinsic factors that are the applied processing in preparation or preservation of dairy products. Thermal treatments and variation in pH value significantly affected foaming and emulsifying properties of milk proteins depending on their intrinsic physicochemical characteristics. On the other hand, emerging technologies in food industry can alter techno-functional properties of milk proteins such as high pressure homogenization, high pressure jet processing, ultrasound, and sonication leading to improved solubility, emulsifying capacity, foaming properties, and thermal stability, thereby enhancing their applications in various dairy and functional food products.

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Chapter 6

Dairy Powders Processing and Characterization

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Abstract

Milk and dairy products are naturally exposed to multiple microbiological, physical and biochemical degradations. Such products require one or more stabilization operations during processing and storing to ensure acceptable sanitary and sensory qualities to the consumer. Several stabilization treatments, such as drying, are commonly applied to dairy products. A successful drying operation requires an in-depth understanding of the physico-chemical changes that occur during the production and storage of dairy powders. Mastering these changes is mandatory to avoid dairy powder degradations, which mainly depend on water dynamics and thermodynamic characteristics of the produced powder. This chapter gives an overview of dairy powder production through the understanding of the powder production principals. A discussion of some essential powder characteristics will be provided. Finally, an overview of the production of camel milk powder will be provided and will be compared with that of cow milk powder.

Keywords: milk, drying, pretreatments, water sorption, glass transition, microstructure

1. Introduction

Milk and dairy products have always been considered as a primary source of nutrients in the human diet. These products are mainly composed of water (up to 90%), proteins, lipids, lactose and minerals. Some of them are subjected to a drying process and are used as food ingredients in various formulations.

Actually, the drying technologies were mainly developed to facilitate the storage and transportation of dairy products as well as to reduce their cost. The ultimate goal of drying is to increase the shelf life of dairy products for a long period of time (up to 2 years) without significant loss of quality [1]. In fact, the transformation of milk into powder allows the elimination of a large amount of water and therefore reduces the milk volume and degradation risks. However, the transition from a liquid state to powder can induce some physicochemical changes in dairy matrix. Indeed, although converting milk and dairy products into powders seems to be advantageous, some technical and physicochemical limitations could be consequent. In fact, it was reported that the biochemical composition of the dairy matrix could limit heat and mass (mainly water) transfers during drying [2, 3]. The presence of sufficient water often makes dairy powders susceptible to multiple microbiological, biochemical and physical

deteriorations [4, 5]. It was reported that some of these deteriorative reactions, such as powder stickiness and caking, lead to a decrease in the powder yield [6, 7], reduce the dryer performance [8], increasing the cost of energy and cleaning/disinfection operation [9, 10] and induce a loss of solubility which negatively impairs consumer acceptability [11]. Therefore, controlling water during the production and storage of dairy powders is crucial to avoid quality loss [1] and to reduce the production cost for the manufacturers [9].

In recent years, there has been a strong interest in the development of drying technologies such as spray drying. This technique has become the most widely used for the production of dairy powders thanks to its paramount advantages such as quickness, no direct contact with the matrix to be dried and ease of implementation [12]. The evaluation of the spray drying impact on the quality of the dairy product requires the knowledge of the physicochemical and compositional differences between the dairy matrices prior to drying [13]. Furthermore, this technological transformation also requires the understanding of the physical (e.g., glass transition and water sorption) and biochemical (e.g., protein denaturation) phenomena that govern the production and storage stability of dehydrated dairy products [14–16].

The understanding of the spray drying mechanism and its impact on the food matrix is the first step to ensure the technological transfer of this dehydration method from one product to another [1]. Several direct and indirect technical indicators were developed to evaluate the dairy powder quality. These indicators are evaluated according to international standardized methods. They constitute fundamental elements to predict the drying parameters, to characterize dairy powders (physicochemical, microbiological and rehydration properties) and to avoid problems due to glass transition, lactose crystallization and dryer fouling [17].

In this chapter, we will first review the classical manufacturing methodology of dairy powders. Secondly, we will provide some fundamental elements on dairy powder characteristics (i.e., the glassy state and the particle microstructure) followed by a discussion of some key indicators (e.g., sorption isotherm and glass transition temperature) to evaluate the quality of dairy powders. Finally, the production of camel milk powder will be reviewed as an example of the technological transfer of dairy powder production.

2. Dairy powders processing

2.1 Milk pretreatments

Before drying, several pretreatments can be applied to the milk and its derivatives (**Table 1**). During the last decades and as a result of technological progress, advanced techniques of membrane separation, purification, enzymatic transformation, demineralization, lactose-reducing, concentration and drying have initiated the appearance of some new dehydrated dairy products. These techniques provide different added values to dairy powders in relation to nutritional solutions for both humans and animals and techno-functional advantages in various foods or pharmaceutical formulations [1].

2.1.1 Membrane separation

Membrane separations are considered as green technologies due to their low cost and energy consumption, high separation efficiency with a large range of particle

Pretreatments	Objectives	References
Heat treatments: Preheating, pasteurization	Inactivation of enzymes (e.g., lipases in whole milk), Inactivation and destruction of pathogenic bacteria	[18]
Skimming, fat standardization and homogenization:	Allow the production of milk powders with different fat contents	[19]
Concentration	<ul style="list-style-type: none"> • Increasing the viscosity and the dry matter content • Reduced energy consumption 	[20]
Membrane separation and purification	Production of various milk protein fractions	[21]
Enzymatic treatment	Production of lactose-free milk	[22]
Pre-crystallization of lactose	Reduce the hygroscopicity of powders	[23]
Formulation	<ul style="list-style-type: none"> • Maltodextrin: preventing sticking and caking of milk powders • Lecithin: instantaneous powder production 	[24, 25]

Table 1.
Milk pretreatments.

sizes, preservation of nutritional and heat sensitive components as well as food properties and recovery of valuable components from dairy wastes [26]. Therefore, they became widely used in the production process of various dairy powders. Techniques such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) were utilized for various purposes including concentration, fractionation of dairy ingredients, adjustment of product content and reduction of the microbial load before the drying process.

Membrane filtration can be utilized for concentration of dairy products before drying [27]. In the case of whey powder production, membrane filtration can be used for whey concentration [28]. After being pasteurized, the whey is passed through a membrane filtration unit (usually NF) for partial ion removal, deacidification, and concentration to obtain whey solution with nearly 15 to 20% (w/v) of total solids content. Afterward, whey can be further concentrated with other techniques and processed to obtain powdered products [29]. Ultrafiltration (UF), reverse osmosis (RO) and nanofiltration (NF) can also be used for milk concentration. They offer the advantages of cost reduction and the possibility to standardize and control the levels of proteins and lactose in dairy powders [30].

Before transforming them into powder, various dairy ingredients can be separated from milk and its byproducts such as whey and buttermilk, using membrane technology, including UF, MF and NF. The UF can be used for the recovery of lactose from whey before its transformation into powder [31]. De Souza et al. [32] used MF, UF, ion exchange, RO and spray-drying for the efficient recovery (74%) and purification of lactose from whey (99.8%). Furthermore, membrane techniques such as UF can be used for the recovery of whey proteins before their transformation into powder. Additionally, membrane filtration techniques can be used to produce high-protein dairy powders [33]. Prior to drying, skim milk produced directly after cream separation can be passed through a series of membrane filtration stages. Firstly, MF ($\geq 1 \mu\text{m}$) can be used to eliminate microorganisms or remaining fat, followed by ultrafiltration to keep micellar casein and whey proteins [34]. Wedel et al. [35] reported also the

possibility of using UF to eliminate microbial cells and spores before drying in order to obtain low-microorganism dairy powders.

2.1.2 Lactose crystallization

After concentration and prior to the drying step, lactose crystallization in dairy products is very important in order to decrease caking and enhance powder quality [29]. The crystallized form of lactose is more stable during the drying process, and it gives less hygroscopic powder with better yield and physical stability [36]. Crystallization of lactose is crucial before drying, especially for products rich in lactose like whey powders [30]. This is supported by some research studies that demonstrated the importance of lactose crystallization in preventing dairy powder caking and stickiness [37].

Lactose crystallization consists of two steps which are nucleation and crystal growth [38]. During this process, a crystallization tank is used to transform lactose from the amorphous to the crystalline form which is less suitable to phase changes during drying at high temperatures and gives lesser sticky particles [29]. The concentrate is seeded with fine lactose crystals, which initiate crystallization by acting as nuclei [30]. Another reported method is the rapid crystallization of lactose in milk powders obtained after spray drying and before their storage in a fluidized bed dryer/crystallizer [39].

The effectiveness of lactose crystallization is highly affected by the composition and the mineral content of the product to be dried. In general, elevated mineral content such as calcium and high concentration of lactic acid can inhibit and retard lactose crystallization [40]. Therefore, it is very important to adjust the content of these components before drying using some techniques like membrane filtration. Other factors to be controlled include viscosity, pH, time, temperature, lactose crystal growth and nucleation, lactose supersaturation and crystallizer design [29].

2.1.3 Instantization

Single stage drying process of dairy products may generate dusty and non-instant powders that have high bulk density and bad rehydration properties. These problems can be solved through instantization which can reduce the bulk density and improve wettability and sinkability of particles to obtain dairy powders that can instantly be dissolved and well dispersed in cold water. Instantization can be generally realized through agglomeration and in some cases by combining agglomeration with lecithination (involves the addition of lecithin which is known for its surfactant properties to powders), especially for high-fat dairy powders [30]. Both instantization methods were tested for improving the physical and wettability properties of skim milk powders, and results showed that combining these two methods gave better results [41].

Agglomeration is the process of raising particle sizes of primary powders by bringing together individual particles to form a cluster-like structure in which the individual particles can still be distinguishable. This increase in particle size results in an increase in powder flowability. The structure of agglomerated particles is characterized by higher volumes of interstitial air in the form of pores and capillaries. This structure reduces the bulk density of powders and facilitates the penetration and movement of water throughout powders *via* capillary action during rehydration [33].

Agglomeration can be used for enhancing the rehydration properties such as wetting and physical properties such as flowability of dairy powders. It can ameliorate the properties of high-protein powders (whole milk, skim milk and fat-filled powders) as well as low-protein dairy powders (whey protein isolate powder, whey protein concentrate powder, casein-dominant powder, etc.) [42]. Agglomeration can be achieved through various techniques including spray dryer agglomeration, fluidized bed agglomeration, shear agglomeration, extrusion agglomeration, roller compaction agglomeration and steam jet agglomeration [33]. However, fluidized bed agglomeration remains the most commonly used agglomeration method for improving the physical and rehydration properties of dairy powders according to numerous studies.

2.1.4 Concentration

In most cases, milk and dairy products are concentrated up to 50–60% in total solids before being dried [17]. This step has several scientific, technological and economic advantages [20]. Indeed, concentration could limit bacterial multiplication by increasing the viscosity and the concentration of milk minerals as well as decreasing the water activity and the pH [20, 43]. In addition, this process induces an increase in powder particle size, which, consequently, impacts some physical properties of dairy powder such as density and powder flowability [44].

Besides, it was reported that the drying operation is expensive for powder manufacturers. The profitability of the drying operation is generally justified only for large production capacities ranging from 10×10^3 to 50×10^3 kg of raw milk per day [45]. Some authors found that concentration leads to a reduction in energy cost by a factor of 10–30 times of powder processing [46]. Currently, vacuum evaporation and reverse osmosis are two major concentration technologies commonly used in the dairy industry. A comparison of these two techniques is provided in **Table 2**. Depending on the specificities of the dairy matrix and the cost of the product, the manufacturer can use one of these two technologies.

2.2 Spray-drying

Drying operation consists of putting in contact a wet droplets with a hot and dry gas. This system assumes that a heat and mass exchange will be established between the two media. In most dryers, the ambient air is used as a dehydrating medium and acts as a carrier for the removed water. The main objective of the drying operation is to obtain a dairy powder with a reduced water content (3 to 5%, w/w) and a water activity (a_w) around 0.2 [49]. At this water activity, dairy powders become microbiologically and biochemically stable. Therefore, preventive measures should be put in place, especially for the control of relative humidity and temperature in the storage environment for milk powders. Generally, concentrated milk (55% total solids) is preheated again to a temperature of 45°C before drying. This preheating ensures the microbiological stability of the concentrated product [50], decreasing the viscosity of the concentrated product [18] and improving drying kinetics [51].

Several types of spray dryers are available to the manufacturers. These dryers are mainly classified according to the number of effects and stages as well as the atomization technology (centrifugal or pressure) [18], the mode of hot air entry (co-current, counter-current and mixed flow) and the geometry of the atomization chamber [45, 50].

Technology and principle	Advantages	Disadvantages	References
Reverse osmosis: Membrane filtration (porosity of 10–3 to 10–4 μm , with a pressure of 30–60 bar).	<ul style="list-style-type: none"> • Preserves the quality of the product. • Energy consumption 2 to 10 kWh/ton of water. • Operating temperature 10 to 60°C. • No water phase change. • Maximum total solids content: 20 to 25% (w/w). 	<ul style="list-style-type: none"> • Rapid fouling of the membranes. • High installation and maintenance costs. • Limited nature of the products (10 to 12% dry matter and fat free). 	[45, 47, 48]
Vacuum Evaporator: Boiling water in a partial vacuum.	<ul style="list-style-type: none"> • High evaporation capacity. • Energy and maintenance costs amortized (a minimum of three effects). • Operating temperature from 45 to 70°C. • Energy consumption 700/n kWh/ton of water (with 'n' number of effects). • Maximum total solids content: up to 60% (w/w). 	<ul style="list-style-type: none"> • Risk of soiling and over-concentration. • High installation cost. 	[17, 18, 20]

Table 2.
Comparison between concentration techniques.

3. Structural characteristics

3.1 Glassy state of dairy powders

Dairy products contain an important amount of lactose. In fact, lactose is a natural diholoside composed of a β -galactose molecule linked to either an α -glucose or β -glucose molecule *via* a $\beta(1\rightarrow4)$ glycosidic bond, resulting in α -lactose or β -lactose, respectively [52]. Depending on the used concentration process, the evolution of lactose state may differ. Indeed, in contrast to slow concentration (e.g., evaporation), the crystallization of lactose is limited by the time factor during spray-drying. Since the latter process is a very quick concentration process, the concentration of lactose increases rapidly without changing the physical state of the lactose. The resulting matrix then remains in an amorphous non-crystalline state called glassy or vitreous state [53, 54]. Indeed, a rapid elimination of water prevents the molecules from crystallizing and favors maintaining of their amorphous state.

In reality, the glassy state is a liquid state showing a solid-like behavior. In fact, a material in a glassy state behaves as a brittle solid (weakly ordered) with no crystalline structure and almost no molecular diffusion (less than $10^{-24} \text{ m}^2 \text{ s}^{-1}$ at 20°C) [54, 55]. This behavior is due to the low molecular mobility, which is a consequence of the very high viscosity of the dehydrated matrix ($> 10^{12} \text{ Pa}\cdot\text{s}^{-1}$ at 20°C) [23, 56, 57].

The vitreous state is a metastable state with relatively slow evolution which may last a long period of time [58]. In this state, molecules generally lose their translational mobility and only retain limited rotational and vibrational mobility. In this configuration, the molecules are thermodynamically unstable (non-equilibrium state) with high hygroscopicity [55, 59]. If the conditions of stability of the glassy

state (temperature and/or humidity) are not respected, the molecules are likely to crystallize (most stable thermodynamic state) [56].

3.2 Structure and microstructure of a dairy powder particle

During drying, milk droplets lose moisture and solidify. The evolution of milk powder particles involves the formation of a cracked crust and vacuoles as a result of the evaporation of water. **Figure 1** shows the microstructure of milk powder containing different fat content. The spray-dried milk particles show a spherical shape, a collapsed structure (**Figure 1**) and a homogeneous wrinkled particle surface [60].

During the formation of the milk powder particle, a concentration gradient of the milk components is created. Two mechanisms are known to describe the transport of the components to the surface. In the first mechanism, surface-active compounds, mainly protein, adsorb easily at the air/water interface and migrate to the surface of the powder as the water is evaporated. In this case, fats, lactose and minerals are believed to be in the inner part of the particle [61]. Kim et al. proposed a second mechanism based on the solid/solute segregation system [62]. As water evaporates from the center of the particles to the surface, diffusion of solids (proteins, lipids, lactose and minerals) to the center of the particles is observed. As a result, the outer surface of the droplet becomes enriched with large molecules such as fat globules and proteins (casein micelles). The transport velocities of these components in the droplets during drying are strongly dependent on the concentration gradients and the values of their diffusion coefficients [63]. Gaiani et al. indicated that a combination of these two mechanisms is more likely possible. Indeed, during the first steps of drying, surface-active compounds migrate to the surface followed by the diffusion of solutes from the surface to the sprayed droplets inner part. This combination could have some limits that are linked to the drying kinetics as the surface could rapidly solidify and solutes migration could be stopped [64].

3.3 Dairy powder characterization

3.3.1 Moisture content and water activity

After drying, residual water can be detected in dairy powders and constitute their moisture content. However, the knowledge of the amount of this water is not

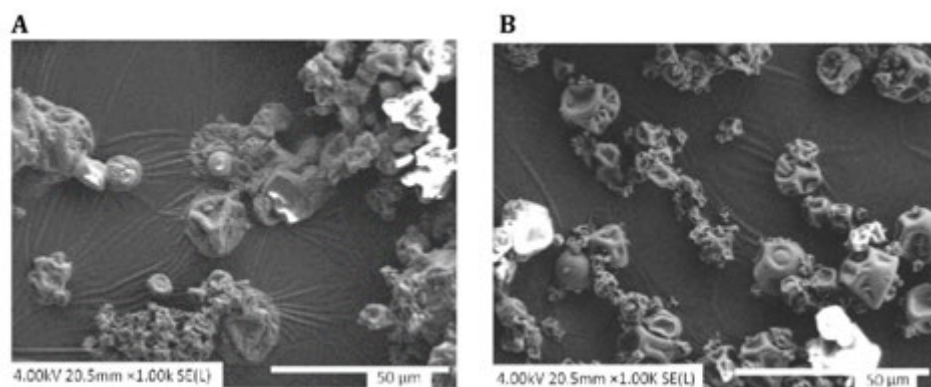


Figure 1. Microstructure of a milk powder. (A) Particles of skim milk powder. (B) Particle of milk powder (27% of fat).

sufficient to assess the stability of dairy powders during storage. Indeed, a sufficient quantity of residual water can induce deteriorative physicochemical reactions and microbiological growth. The notion of water activity was developed to consider the interactions of water with the other components of the dehydrated matrix [65].

The moisture content as well as the water activity are strongly related to the thermodynamic properties of the drying air, especially its relative humidity [49]. In the case where the relative humidity of the drying air is not controlled, the amount of water in the powder can vary for the same drying temperatures. Understanding the enthalpic diagram of moist air called Mollier diagram, allows to choose the drying temperatures and predict the water activity of the milk powder produced. For good storage, water activity should be less than a critical water activity of 0.37 [57]. However, it is recommended to produce powder with a water activity of 0.2 for optimal conservation. At this water activity, the water content varies from 3 to 5% (w/w) depending on the nature of the dairy powders [21].

3.3.2 The sorption isotherm

Dairy powders are thermodynamically unstable. Indeed, they are generally hygroscopic and tend to adsorb moisture from storage air [57]. When the storage conditions are not respected, the water activity can drastically increase. Exceeding a critical activity, physical changes, such as lactose crystallization, could occur. To determine this critical activity, an accurate evaluation of the sorption isotherm for each produced dairy powder should be conducted. Indeed, the sorption isotherms are known to describe the equilibrium relationship (at constant temperature and pressure) between water content and water activity of food and dairy powders [66, 67]. The understanding of sorption isotherms can provide guidelines to control the drying process and to determine the storage conditions through the analysis of the desorption isotherm and adsorption isotherm, respectively [1].

Several factors could influence the adsorption of water by dairy powders (**Table 3**) and, consequently, the adsorption isotherms. These isotherms are described as a sigmoid curve showing a breaking point (**Figure 2**) corresponding to the apparent conditions of lactose crystallization [71]. This phenomenon occurs at a water activity

	Factors	References
Water adsorption	The quantity and hydrophobicity of proteins	[14, 68]
	The quantity and nature of minerals	[15]
	The quantity as well as the biochemical (hydrolyzed or not) and physical state of lactose (amorphous or crystalline)	[14, 67]
	The particle size and surface composition of milk powder particles	[69]
	Humidity, temperature and storage time	[70]
Glass transition temperature	Temperature and water dynamics during storage	[71]
	Matrix Composition: water content, protein content (quantity, size and hydrophobicity) and lipid content	[14, 72]
	Molecular weight of polymers: Tg increases with increasing molecular weight	[73]

Table 3. Factors influence the adsorption of water and the glass transition temperature of dairy powders.

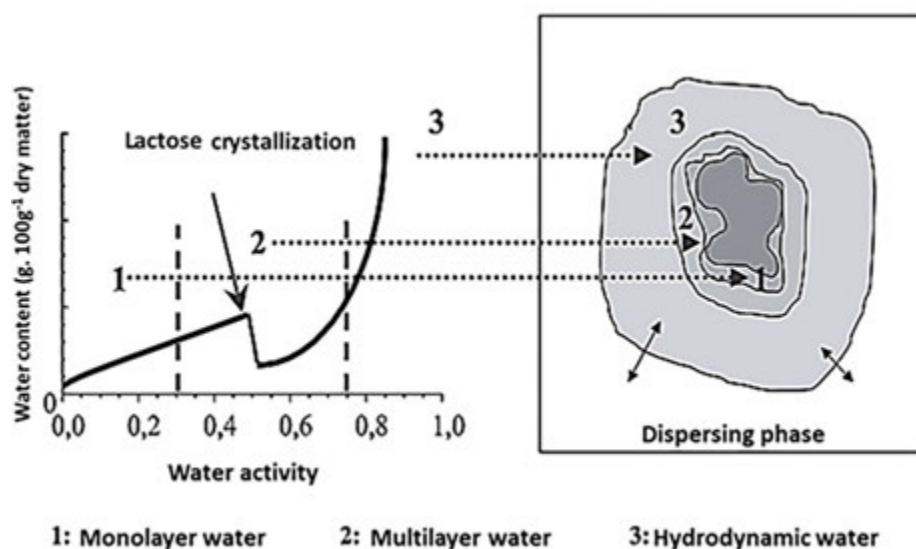


Figure 2.
Adsorption isotherm of a skimmed milk powder. Adapted from Shcuck et al., [52].

ranging from 0.37 to 0.7 and is characterized by a strong decrease in adsorbed water content following crystallization of amorphous lactose as α -lactose monohydrate [7, 68, 74]. Several authors have suggested dividing the adsorption isotherm of dairy powders into three distinct parts (**Figure 2**). In the first part, a gradual increase of water adsorption up to 0.2 to 0.3 of water activity (low moisture content) could be observed. The adsorbed water in this area is called monolayer moisture [75]. The proteins (caseins and serum proteins) are suggested to be the main water absorbers through water-protein interactions [68, 74]. In the second part, a quick increase of adsorbed water is observed (up to 0.6 to 0.7 of water activity). A breaking point could occur as a consequence of a water release describing the change in physical state of the amorphous lactose, which absorbs water until crystallization [68, 76]. Beyond the breakpoint, water adsorption is resumed as milk minerals have a strong influence on water adsorption in this third part [53, 76].

3.3.3 The glass transition temperature

The glass transition is a key concept in food polymer science, and it can be affected by several factors (**Table 3**). The glass transition is considered as a second-order reaction during which state changes could occur, resulting in an important physical alteration of food powders. Indeed, during the glass transition, the system characteristics shifted from the glassy to the rubbery state, during which the matrix lose its viscosity. Such transition is essentially accompanied by two types of changes in physical properties. These changes are related to the rearrangement of the internal structure of amorphous molecules, leading to the modification of the rheological properties (viscoelastic modulus) and the thermodynamic properties (enthalpic relaxation, thermal capacity and coefficient of expansion) [57, 77, 78].

The most important parameter in this concept is the glass transition temperature (T_g). Knowledge of T_g is essential to describe the properties of a material because of its good correlation with its structural and thermodynamic properties [3, 78].

However, it was assumed that the glass transition takes place over a temperature range with an onset T_g ($T_{g_{onset}}$), a midpoint T_g ($T_{g_{midpoint}}$) and an offset T_g ($T_{g_{offset}}$). The range between $T_{g_{onset}}$ and $T_{g_{offset}}$ can vary from 10 to 20°C for amorphous sugars (e.g., lactose) and up to 50°C for high molecular weight polymers [55, 79]. The $T_{g_{midpoint}}$ is considered as the main glass transition temperature (T_g), which is specific for each material [51].

In the lactose/protein matrix (e.g., dairy powders), the glass transition temperature is relatively dominated by the physicochemical properties of lactose. Indeed, at the same water activity, dairy powders have a glass transition temperature approximately equal to that of a lactose-water mixture [7, 68]. At a temperature below T_g , the glass transition in dairy powders can be affected by the presence of water. In fact, water is considered as the main plasticizer in dairy powders. The glass transition temperature of pure lactose is equal to 101°C. At 10% water (pure lactose) or 5% (in milk powder), the T_g of lactose decreases to about 30°C. The water plasticization phenomena can be assessed by measuring the glass transition temperature. Indeed, a decrease in the T_g of hydrophilic molecules is observed as the water activity of the powder increases [59, 80, 81].

In dairy powders, the plasticizing effect of water only concerns lactose or its mixtures with proteins [82]. Approaching a critical water activity, water plasticization results in the softening of the dairy powder, followed by lactose crystallization [53, 68]. Indeed, it was demonstrated that the plasticizing effect of water particularly affects the thermal properties and structural resistance (α -relaxation) of lactose [68, 72]. An increase in water content reinforces the interactions of lactose with water through strong hydrogen bonds. Such interactions lead to an increase in lactose mobility, which results in a decrease in dairy powder's viscosity [59, 77, 83].

It is then important to control the glass transition temperature to avoid certain alteration reactions (**Table 4**) which are initiated depending on the difference between the storage temperature and the glass transition temperature. These reactions lead to a decrease in the shelf life and loss of quality of dairy powders [54, 58].

3.4 Physicochemical characterization

3.4.1 Whey protein denaturation (WPNI)

Several researchers have developed protein quality indicators such as the Whey Protein Nitrogen Index (WPNI) [50, 87]. The accuracy of this indicator can be influenced by several factors such as the feed nature [4], the equipment design [15] and the protein composition and quantity of the dairy product before drying [15, 88]. Indeed,

Consequences	References
Lactose crystallization and water release	[71, 84]
Powder Stickiness on dryer wall	[9]
Powder caking and flow difficulty	[3]
Initiation of the Maillard reaction	[85]
Fat oxidation	[86]

Table 4.
Main consequences of glass transition.

the protein fraction of milk is almost composed of 80% caseins and 20% of whey proteins. It is widely known that the latter protein group was the most sensitive to technological treatments, especially thermal operations, even for a short period of exposure. In fact, during heat treatment ($> 60^{\circ}\text{C}$), whey proteins are firstly denatured and then irreversibly aggregate, leading to the formation of insoluble precipitates [89, 90].

The production of dairy powder includes a set of heat treatment operations that can modify the quality of milk proteins such as pasteurization and drying [15, 91, 92]. Thus, whey protein denaturation can be used as an indicator of the intensity of the drying process through the assessment of the WPNI [20], allowing the manufacturer to classify their dairy powders (**Table 5**). However, this indicator is mainly based on the loss of cow β -lactoglobulin [95], which may mislead the WPNI results for other milk obtained from other breeds that lack β -lactoglobulin.

3.4.2 Surface free fat content

Fats in dairy powders could exist in the free form at the particle surface (the outer surface and the vacuoles, pores and cracks surfaces) [18, 96]. In most cases, a high surface-free fat content SFC in dairy powders is not desired, except in the chocolate industry [97]. Indeed, SFC is often considered as an indicator of fat instability [98]. A high free fat content enhances the fat oxidation in milk powder [18] and changes in the color of the powder [86]. Moreover, fats at the powder surface increase the wetting time [99, 100] and affect the flowability of the powder [101, 102].

3.4.3 Powder densities, flowability, and particle size distribution

One of the main objectives of dairy powders production is to facilitate the handling and to reduce the transportation cost of some dairy products. Moreover, certain powder characteristics, such as flowability and rehydration, are strongly influenced by the density of the dairy powders [18, 20].

To achieve these goals, dairy powders should have a high density [4, 87]. Density is a complex characteristic and is influenced by several factors including the composition, the viscosity and the foam-ability of the concentrate before drying [20]. After drying, the particle size, the amount of occluded and interstitial air [50], the particle shape of the milk powder [4], and the physical state of lactose (amorphous or crystalline) [18] are also involved in determining the powder density.

Class	WPNI ($\text{g N}_2 \cdot \text{kg}^{-1}$)	Examples of application
Extra low-heated powders	> 8.0	• Reconstitution and standardization of dairy products
Low heated powders	$6 \text{ à } 7.99$	• Ingredient in ice cream production • Fortification of cheese and yogurt
Medium heated powders	$4.5\text{--}5.99$	Chocolate and confectionery
Medium-high heated powders	$1.51\text{--}4.49$	Reconstituted condensed milk
High heated powders	< 1.5	Ingredient in bakery products

Table 5.
Thermal classification of dairy powders and their potential applications [15, 18, 93, 94].

Flowability	Carr's Index (%)	Hausner Ratio
Excellent	0–10	1.00–1.11
Good	11–15	1.12–1.18
Acceptable	16–20	1.19–1.25
Passable	21–25	1.26–1.34
Low	26–31	1.35–1.45
Very low	32–37	1.46–1.59
Extremely low	> 38	> 1.60

Table 6.
Classification of powders according to their flow index [103].

The powder flowability is a complex measurement that describes the ability of dairy powders to move freely during handling. The flowability is often influenced by the lactose crystallization level, the caking of the powder [3], the surface free fat content [4] as well as the shape and the size distribution of the particles [18]. The particle size distribution is an important characteristic of dairy powder quality. This parameter is mainly influenced by the drying method, the concentration and viscosity of the concentrate as well as the droplet size before drying [20]. Some indicators, such as Carr's index (compressibility index) and Hausner's ratio, have been proposed to classify powders based on their flowability (**Table 6**).

3.4.4 Rehydration properties

Rehydration describes the process of reconstituting the powder in water and is considered as the key characteristic for subsequent use of all food powders. This process is generally evaluated by the measurement of the insolubility, the wettability and the dispersibility indexes [20].

The insolubility index reflects the denaturation of proteins and the formation of insoluble complexes [104, 105], while the ability of the powder to be wetted by water without stirring is evaluated through the determination of the wettability index. Generally, a powder with high wettability has a wettability time of less than 3 min [17]. The dispersibility index describes the ability of the powder to disperse and disintegrate in water. This parameter is a good indicator of the instantaneity of milk powder [20]. A dispersible powder has a dispersibility index greater than 90% for skim milk powder and 85% for whole milk powder.

4. Camel milk powder production

Camel milk is one of the primary food stuff for nomads in arid and semi-arid regions. In traditional pharmacopeias, this milk is described for its nutritional and medicinal properties. Nowadays, the trading of raw or frozen camel milk is considered as an emergent international business. However, the transport and freezing operations are very expensive.

To overcome these problems, converting camel milk into powder could represent a valuable solution as it is already done for cow milk. However, such transformation could be very challenging due to the composition and the physiochemical of this

milk. Actually, the physicochemical analysis of camel milk shows that the protein and lactose amounts are likely identical to cow milk with slightly higher ash content. However, the protein composition was totally different. Indeed, analysis of camel milk proteins showed higher β -casein (47%) and lower κ -casein (3.5%) content [13]. The particularity of camel milk whey is the lack of β -Lactoglobulin and the overexpression of α -lactalbumin. Such protein composition may impair the powder particle formation and the transport of these compounds to the particle surface, which may change the powder properties [106].

Studies on the production of camel milk powder indicated that camel milk exhibited similar thermodynamic behavior during drying compared to cow milk. Overall, camel milk powder presented a higher bulk density than cow milk, showing comparable solubility [107]. Besides, it was indicated that camel milk proteins showed better thermal stability during drying. This stability is mainly due to the absence of β -lactoglobulin and the low initial content of free thiol (SH) groups [13]. Analysis of Scanning electron microscope (SEM) and Confocal Laser Scanning Microscopy (CLSM) micrographs of camel milk powder particles showed a spherical shrunk structure with relatively high fat and lactose content at their surface. The analysis of adsorption isotherms and glass transition temperature shows that beyond the glass transition, lactose in camel milk was less stable against crystallization. Lactose in camel milk completely crystallized at a critical water activity of 0.60 at a temperature of 25°C (instead of 0.70 for cow milk powder). This low stability was linked to the exposure of a high lactose content on the surface of camel milk powder particles [13]. However, analysis of moisture sorption isotherm indicated that at low water activity, camel milk powder showed a better storage ability when the glass transition conditions are respected [106, 108].

5. Conclusion

Milk and its derivatives are water-rich products which makes them perishable with very short shelf life. To solve such problem, dairy products generally undergo stabilization treatments such as drying. Several drying methods have been developed for the production of dairy powders especially the spray drying. This technique allows rapid evaporation of water and the production of a stable powder for a long storage period (up to 2 years). However, maintaining this stability is a critical step and need to be evaluated. Several indicators have been proposed to assess the quality of dairy powders. Most of these indicators are likely related to water dynamics and its relationship with the stability of dairy powders. Indeed, water influences directly or indirectly the speed of physical and biochemical alterations. Currently, the available knowledge of milk transformation is generally derived from the processing of cow's milk. The transfer of this knowledge to other types of milk such as camel milk constitutes, in most cases, a scientific and industrial challenge. This chapter offers a guideline to characterize dairy powders through the understanding of the basics of dairy powder production and characterization.

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Goat Milk: Composition and Quality

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Abstract

Goat milk has become a profitable dairy farming activity worldwide, due to its potential benefits for human nutrition and health. This chapter provides a review of the nutritional, physicochemical, and microbiological aspects of goat milk. It highlights the high nutritional value of goat milk, which is rich in essential nutrients such as proteins, carbohydrates, fats, vitamins, and minerals. Additionally, the chapter explores beneficial attributes such as its less allergenic protein composition, digestibility, and fatty acid profile. Furthermore, it examines the microbiological quality of raw goat milk, emphasizing the importance of good hygiene practices, cooling, and proper management to prevent contamination by potential pathogens such as *Salmonella* spp. and *Staphylococcus aureus*. Additionally, the chapter discusses the functional food properties of goat milk, including its use in probiotic-rich products like yogurt and kefir. This review underscores the significance of goat milk as a nutritious, digestible, and health-promoting food with an increasing role in the global dairy market.

Keywords: goat, milk, quality, microbials, safety

1. Introduction

Goat milk and its dairy derivatives, such as yogurts, ice cream, and cheeses, began to be appreciated not only for gastronomic quality, but also for its nutritional facts. Goat milk has become an alternative to cow's milk for those allergic consumers due to the lower levels of α -s1 casein [1, 2].

Goat milk production plays a vital role in the economies of developing countries, offering sustainable livelihood alternatives, particularly in regions with limited environmental and socioeconomic resources. It can generate income and create employment opportunities for both small and large producers. The growing demand for goat milk and its derivatives is closely tied to higher per capita income and the increasing interest in healthier eating habits [3]. Over the past 50 years, global goat milk production has more than doubled, with a projected increase of approximately 53% by 2030 [4]. In 2017, global production was estimated at 18.7 million tons, with India leading production, followed by countries like Bangladesh, Sudan, Pakistan, France, and Greece [5]. This growth is partly due to the characteristics of goat milk, which, compared to cow's milk, is more digestible and has a lower potential for causing

allergies [6]. Additionally, goats play a key role in rural economies, especially in arid and semi-arid regions, where their resistance to adverse climates and their ability to consume plants not utilized by other animals make them essential for diversifying production [7]. In many developing countries, goat farming occurs in diverse systems, with extensive and intensive systems being the most common, requiring minimal use of external inputs, which is an advantage in resource-limited areas [5].

Although goat milk production is significant, it faces challenges such as a lack of public investment, low productivity due to inadequate management practices, and limited access to financial resources, especially for small producers. These challenges are further exacerbated during dry periods, which reduce the availability of forage, forcing producers to cut back on the number of animals. However, the goats' adaptability to these harsh conditions makes them a resilient solution for food production in times of scarcity [6, 8]. On the global stage, the commercialization of goat products faces infrastructure limitations and trade barriers, but there are promising prospects, especially in the Middle East, a major importer of goat meat and live small ruminants. Overcoming economic challenges and investing in more efficient technologies could improve productivity and sustainability in goat farming, benefiting small producers and expanding the availability of goat milk derivatives [9].

As a consequence of dairy goat farming emerging as a profitable alternative to agribusiness, research on the physicochemical and microbiological properties of goat milk is essential for the success of goat dairy production, trade, and safety. This chapter offers a comprehensive review of the key physicochemical and microbiological aspects of goat milk and its dairy derivatives.

2. Goat milk: Nutritional, physicochemical, and sensory aspects

According to the World Health Organization (WHO) [1], milk should be part of the daily diet for children and adults of all ages. Goat milk is considered a high-quality food with significant nutritional value, containing essential elements, such as, high biological value proteins, carbohydrates, fats, vitamins, and minerals, making it an important food for human nutrition. Studies state that the daily consumption of at least 1 liter of goat milk can meet one-third of an adult's daily needs, as it has higher concentrations of calcium, phosphorus, potassium, and magnesium compared to cow's milk. Its milk also has higher levels of sodium, phosphorus, potassium, and vitamins including thiamine, vitamin A, choline, riboflavin, niacin, and biotin when compared to human milk. However, goat milk has lower levels of vitamin B6, B12, vitamin K, ascorbic acid, folic acid, and pyridoxine (**Table 1**) [2].

The composition of goat milk can vary depending on several factors like breed of the stock, its diet (which is a determinant factor for milk quality), by the climate, health, lactation stage, reproduction, and milking technique, as well as the subsequent handling of the product. In general, the average composition of goat milk nutrients found in the literature ranges from 0.70 to 0.85% mineral salts, 2.6–4.1% high biological value protein, 3.0–5.6% fat, and lactose ranging from 4.1 to 4.3% [12].

In addition to the nutritional benefits of goat milk, it is recognized as a food with great market potential due to its high digestibility and lower tendency to cause allergies compared to cow's milk. This makes it an ideal option for individuals with gastric issues and those who are allergic to cow's milk. Its high digestibility is related to the fat globules and the profile of fatty acids present in its composition. The fat globules are mostly smaller than those from cow's milk, which allows greater surface area for contact with

Component	Approximate amount (per 100 mL)
Calories	69 kcal
Protein	3.1 g
Fat	4.1 g
Carbohydrates	4.7 g
Sugars (lactose)	4.3 g
Calcium	134 mg
Phosphorus	95 mg
Potassium	204 mg
Magnesium	14 mg
Vitamin A	33 µg

Adapted from: USDA Food Database and Kumar et al. [10, 11].

Table 1.
Nutritional composition of goat milk (per 100 mL).

digestive enzymes, facilitating their action on the fat, making easily degradation and better absorption by the body, which explains its high digestibility rate [13]. The profile of fatty acids is composed of short and medium chains, which includes higher proportions of butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), and capric acid (C10:0), acids that do not only contribute to the characteristic aroma and flavor of goat milk but also aid in digestion and absorption by the body due to their molecular structure [14, 15].

Another important feature of goat milk is its protein content. Just like fat, protein also affects digestibility and is one of the factors that allows many people allergic to cow's milk to consume goat milk. The protein in goat milk is found in the form of casein (α 1-casein, α 2-casein, β -casein, and k-casein), with α -lactalbumin, β -lactoglobulin, serum albumins, and immunoglobulins being the soluble proteins [16]. Studies indicate that α S1-casein, found in greater amounts in cow's milk, is the protein responsible for allergic reactions in people sensitive to cow's milk. However, the proportion of α S1-casein in goat milk is significantly lower than in cow's milk, which facilitates the formation of smaller curds that are more easily broken down by proteolytic enzymes. Also, casein micelles in goat milk typically range from 100 to 200 nm in diameter, smaller than those in cow's milk, which usually range from 200 to 300 nm. This smaller size of casein micelles in goat milk aids in better digestion and absorption (**Table 2**) [18, 19].

Scientific evidence has further elucidated that goat milk can be considered as an excellent dairy alternative to improve human health especially to the people suffering from lactose intolerance, as it contains less lactose than cow's milk. Also, acidity tends to increase due to the high microbial activity on lactose, which undergoes hydrolysis by enzymes, leading to the formation of lactic acid [11]. Acidity serves as an indicator of quality standards, as it allows the identification of the quality and the state of milk preservation. The quality of the initial raw milk directly impacts the final product's quality and shelf life. However, unlike the raw goat milk, pasteurized milk or after thermal treatment stabilizes its acidity. This microbial load can undergo changes both regarding the storage time of raw milk, which can promote microbial growth and improper hygiene procedures during subsequent milk processing [20].

Goat milk also differs in color from cow's milk because goats have the ability to convert all the β -carotene in their diet into vitamin A, meaning that the milk does not contain this

Proteins	Goat milk	Cow milk
Total Proteins	31 to 36 g/L	32 to 35 g/L
<i>Caseins</i>	60–80%	80%
α1-casein	15–26%	38–56%
α2-casein	10%	11%
B-casein	53–64%	25–33%
k-casein	8–15%	8–15%
<i>Whey Proteins</i>	20%	20%
β-lactoglobulin	12–16%	7–12%
α-lactalbumin	6–10%	5–10%

Adapted from: Verruck et al. and Delgado et al. [3, 17].

Table 2.

Protein content of goat and cow milk (per 100 mL).

pigment, thus appearing white and being considered a rich source of vitamin A. Cow's milk, on the other hand, has a more yellowish color because cows cannot convert ingested β-carotene, which remains in the milk [21, 22]. Another notable sensory aspect is the characteristic smell and taste of goat milk, which is often described as "hormonal." This can be attributed to a combination of factors, including the lipid composition (particularly the short-chain fatty acids), the presence of hormones during lactation, goat's diet, as well as the management and hygiene conditions. During the reproductive cycle of goats, hormone levels like estrogen and progesterone may increase. These hormones can, in certain cases, be excreted in the milk, especially if the goat is pregnant or lactating after estrus. The increase in these hormones can affect the taste and smell of the milk, resulting in a "hormonal taste" perception. The fact that goat milk contains caprylic acid and caproic acid, which contributes to a stronger odor, is typical of the "hormonal taste." If the taste or smell of goat milk is very strong, it may be helpful to investigate the management of the goats, their diet, or the storage time of the milk, as these factors can either mitigate or intensify these aromas [11].

Goat milk has become a widely used as raw material to dairy products. Although the amount of goat dairy products is commercialized in smaller quantities compared to cow's milk, its products are increasingly present in the market and gaining consumer popularity. Some of these products include pasteurized milk, UHT milk, powdered milk, ice cream, sweetened condensed milk, yogurt, fermented dairy beverages, butter, cheeses with or without condiments, and more [17]. Currently, goat milk is also considered a functional food as defined by the term, participating in maintaining health, by reducing the risk of chronic diseases, as well as in physiological modifications [23]. Studies already point out the use of goat milk in the production of probiotics that assist in maintaining gut flora, especially in fermented milks, fermented dairy drinks, yogurt, kefir, curd cheese, ricotta, and ice cream, which, due to their composition, promote the growth of bifidobacterial and lactic acid bacteria. When present in balanced quantities in humans, these bacteria are beneficial to health [24–27].

3. Goat milk: Microbiological quality

The microbiological composition of raw goat milk is naturally represented by lactic acid bacteria in species of the genera *Lactococcus* spp. and *Lactobacillus* spp. and

members of the Enterobacteriaceae family. However, it can undergo variations due to extrinsic factors such as climatic conditions during milking, genetic factors, diet, and the health of the goat herd [28]. Goat milk is often associated with the presence of infectious agents or toxins that pose a public health risk, including *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., enterotoxigenic strains, *Escherichia coli* Shiga toxin-producing strains (STEC), *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella* spp., and *Cronobacter* spp., *Mycoplasma* and *Mycobacterium* [29–31].

The standard of identity and quality of goat milk is defined by milking management practices, cooling at the farm, transport, physicochemical parameters, microbiological factors, and somatic cell count. Among these parameters, microbiological quality is a factor that directly impacts the raw material and its derivatives and serves as an indicator of hygiene and milking practices, as the presence of microorganisms results in the loss of sensory quality, durability, and the effectiveness of processing steps, making the product unsafe for consumption.

Therefore, by controlling and ensuring the quality of the initial raw material throughout the management, thermal treatment, transportation, and storage stages until the milk reaches the final consumer are crucial. Regulations set microbiological criteria as quality indicators, such as counting viable mesophilic and psychrotrophic aerobic bacteria, total enterobacteria, coagulase-positive *Staphylococcus* counts, detection of *Salmonella* spp., and *Listeria monocytogenes* [32].

3.1 Lactic acid bacteria and yeasts with probiotic potential

Lactic acid bacteria have an important impact on the composition and quality of goat's milk, contributing to both microbiological and sensory qualities, as well as to the safety and health of consumers of goat milk. They are crucial for the production and fermentation of cheeses, yogurts, and other dairy products. They are coadjutants of milk preservation and can act as probiotics that generate benefits for intestinal health, improving digestibility, especially in relation to lactose. Probiotic effects also include the reduction of serum cholesterol levels, by bile salt hydrolase (BSH) activity of some lactic acid bacteria [33, 34]. Bacterial species of genera *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Enterococcus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* and yeasts of *Candida* and *Diutina* genera are commonly found in goat's milk and its fermented products. *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, and *L. rhamnosus* are applied as probiotics and as adjuvants for the creamy texture of yogurt and fermented dairy products. Other bacterial species with probiotic potential have been described are the *Enterococcus faecium*, *E. faecalis*, and *Lactococcus lactis* that presented the ability to produce bacteriocin with complete inhibition activity against *Listeria monocytogenes* and *Staphylococcus aureus*, tolerance to bile salts and simulated gastrointestinal environments, adhesion capability, and safety [35]. *Candida tropicalis* and *Diutina rugosa* were reported resistance to pH 2.5, 0.3% bile salt, auto-aggregative, and hydrophobic properties of proper criteria as probiotics [36].

3.2 Mesophilic aerobic bacteria

Mesophilic bacteria are microorganisms capable of growing at temperatures of 35–37°C, and their count reflects inadequate hygiene practices, such as insufficient cleaning of goat stalls, handlers' hands, and equipment used during milking. This suggests that the milk may be contaminated with microorganisms from the environment, compromising its quality [37]. These bacteria are often responsible for milk

spoilage, affecting its sensory characteristics and reducing the shelf life. A high presence of these bacteria can accelerate the spoilage process, making goat milk unsuitable for consumption earlier than expected [38].

The mesophilic bacteria count in raw goat milk should not exceed 10^5 CFU/mL, as established by Brazilian's standards for milk microbiological quality control [39]. Limits for total mesophilic bacteria plate count of raw milk by The European Commission [40] defined limit of 100,000 CFU/mL. Whereas in New Zealand [41] the limit is 300,000 CFU/mL [41], and Canadian standards limit is 50,000 CFU/mL mesophilic bacteria count [42]. When the mesophilic bacteria count exceeds, this limit will result in sanctions for producers and health risks for consumers. Additionally, it can impact the effectiveness of pasteurization processes. If the mesophilic bacterial load is high, higher temperatures or longer pasteurization times may be required to ensure product safety, which may not be economically viable. The effectiveness of goat milk refrigeration can also be affected by a high initial count of mesophilic bacteria. These bacteria can continue to multiply, leading to an increase in the total microbial load of goat milk during cold storage [28].

3.3 Psychrotrophic aerobic bacteria

Psychrotrophic bacteria are among the primary groups responsible for spoilage in raw goat milk and other dairy products. These microorganisms predominantly belong to the genus *Pseudomonas* spp., and *Pseudomonas fluorescens* are the most common species isolated from milk samples. Psychrotrophic bacteria are able to produce heat-resistant enzymes, including proteases and lipases, which are active at low temperatures. These enzymes can break down milk fat and proteins, leading to the development of off-flavors and spoilage events during preservation at low temperature [28, 43]. Cold storage may mask the impact of contamination caused by poor hygiene practices on the farm, which does not prevent bacterial growth entirely. In fact, although the total microbial count may remain low and within acceptable limits during cold storage, psychrotrophic bacteria levels approach the critical threshold of 5.0 log CFU/mL within few days and act as an important factor of milk degradation [43].

3.4 Total and thermotolerant coliforms

Total coliforms is a group of microorganisms belonging to the Enterobacteriaceae family, consisting of the genera *Escherichia*, *Enterobacter*, *Citrobacter*, and *Klebsiella*, capable of fermenting lactose and producing acid and gas when incubated at 35–37°C for 24–48 hours. They are important indicators of hygienic-sanitary quality, as their presence in food indicates the level of contamination of environmental origin, such as post-thermal treatment contamination, since they are sensitive to pasteurization temperatures. Thermotolerant coliforms are lactose fermenters that produce gas when incubated at 45°C. Their presence in food indicates fecal contamination, where high levels can cause symptoms like diarrhea and other intestinal disorders. Failures during raw milk transport, especially by inadequate temperatures, can allow the growth of coliforms. Improper storage conditions can also lead to the multiplication of these microorganisms. Another relevant factor is the occurrence of environmental mastitis in dairy goats, which can result in contamination of milk by coliforms and other pathogens. Infected animals can excrete high levels of infectious bacteria in their milk, increasing the risk of contamination [44]. Thus, the presence of coliforms in food reflects that it has undergone inadequate hygiene practices of utensils and

equipment, both during milking and in processing stages, as well as failures during thermal treatment. A lack of access to information or proper training on good management and hygiene practices in milk production can increase the risk of coliform contamination by the milking team and food handlers.

3.5 *Salmonella* spp.

Salmonella spp. is a microorganism of significant interest for public health and the food industry, due to the foodborne infections caused by the ingestion of untreated meat, poultry, eggs, milk, and dairy products, as well as its ability to remain viable for long periods in food, even in frozen foods. The bacteria develop at 37°C but is capable of multiplying at temperatures ranging from 7 to 49.5°C [45, 46]. The presence of *Salmonella* in raw goat milk has significant implications for public health and food safety, potentially causing symptoms including diarrhea, fever, and abdominal cramps. In vulnerable groups such as children, the elderly, and immunocompromised individuals, infection can be more severe and even fatal [47]. Worldwide legislation establishes that *Salmonella* is not allowed in any dairy products. Detection of this bacterium in raw goat milk indicates failures in hygiene practices during milking and processing, requiring improvements in sanitary controls and awareness among producers about good practices.

Raw goat milk can be a source of cross-contamination in derived dairy products, like cheeses, ice creams, and desserts. The presence of *Salmonella* spp. in unpasteurized milk can lead to contamination of these products during processing, increasing the risk of foodborne outbreaks. Strict control of hygiene conditions during milking and proper thermal treatment of goat milk is crucial to prevent *Salmonella* contamination. Pasteurization is an effective measure to eliminate this bacterium, but the consumption of raw milk remains a common practice in some regions, increasing the public health risk [48].

3.6 *Staphylococcus* spp.

The presence of coagulase-positive staphylococci, especially *Staphylococcus aureus*, can have various implications for the microbiological quality of goat milk and human health. Several strains of *Staphylococcus* spp. have the ability to produce enterotoxins (*Staphylococcus* Enterotoxin) that can remain viable in milk, even after thermal processing. Ingesting goat milk and dairy products contaminated with enterotoxins can lead to symptoms like vomiting, diarrhea, nausea, and abdominal cramps, often occurring within hours after consuming contaminated food. In this case, strict control of food temperature is necessary, with refrigeration temperatures kept below 7°C [49]. Thus, proper care during and after milking, along with mastitis control measures, can positively influence the prevention of contamination of raw milk [50].

Additionally, it is important to rigorously control the hygiene of handlers who carry strains of *Staphylococcus* spp. associated with skin and mucosal infections, as well as secretions from these infected individuals can act as a source of contamination for milk during milking and processing. *Staphylococcus aureus* colonizes the skin and mucous membranes of humans and animals and can also be one of the main causes of skin infections, such as boils, abscesses, and cellulitis, and infectious mastitis in goats. Mastitis may be present as subclinical or clinical disease, with redness, swelling, and pain in the affected area, potentially evolving into severe cases with necrotic lesions that can result in the loss of the udder [51].

4. Conclusions

Goat milk is an increasingly significant product in the global dairy agribusiness, offering various nutritional, sensory, and health benefits. Its high digestibility, lower allergic potential, and rich nutrient profile make goat's milk an attractive alternative to cow's milk, particularly for individuals with special dietary needs. However, the safety and quality of goat milk are highly dependent on proper hygiene practices in all handling steps, from milking to final product processing. The microbiological quality of goat milk plays a crucial role in determining its suitability for consumption, and control measures must be strictly enforced to prevent contamination by harmful microorganisms. Understanding these aspects is vital for improving quality of goat milk production, ensuring its safety, and increasing its potential value to dairy derivatives and as a functional food.

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Conflict of interest


Authors declare no conflict of interest.

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Milk Processing and Dairy Products Industry offers a comprehensive overview of the processing and manufacturing stages involved in transforming milk into a wide range of dairy products, from the reception of raw materials to final packaging. It also addresses key aspects of quality assurance and food safety. As a unique and authoritative reference, this book bridges the gap between theoretical foundations and real-world applications. It provides readers with a thorough understanding of the technologies, processes, and quality standards that shape today's dairy industry. This unique book presents the latest research on preservation methods in the dairy industry, covering both thermal and non-thermal technologies, as well as fermentation processes.

The book also explores the physicochemical and microbiological quality of dairy products derived not only from cow's milk but also from other mammalian sources such as goat milk. Each chapter has been carefully crafted by experienced dairy scientists, ensuring both scientific rigor and practical relevance throughout the volume. Topics discussed include:

- Thermal and non-thermal milk processing technologies in the dairy industry
 - Producing and characterizing dairy powders
- Innovative approaches to minimize waste and optimize resource use in milk processing
 - Precision fermentation can create dairy-identical proteins
 - Goat milk's nutritional profile and quality traits
 - Hygiene and compositional quality of raw cow milk

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