

Fermentation intricacies involving dairy milk and their bioactive, health, microbial, and product challenges: A terse review

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Abstract

Fermentation is widely applied to enhance the safety, stability, and functional properties of dairy milk as part of broader milk processing and hygiene controls. This concise review examines the intricacies of dairy milk fermentation, focusing on microbial activity, bioactive chemical aspects, and product quality/safety. Bioactive peptides derived from fermentation-driven acidification have been shown to kill bacteria, contain antioxidants, lower blood pressure, and modulate the immune system, thereby enhancing microbiological safety and shelf life. However, there are key aspects, from milk composition, through culture performance, to processing circumstances that can affect safety and uniformity. Besides, bioactive molecular characterization, indigenous starter culture confirmation, and probiotic efficacy are required to enhance the success of dairy milk fermentation. To ensure high-quality, safe, and stable fermented dairy products, the process of fermentation science and risk-based regulatory frameworks must not be overlooked. Moreso, enhanced regulations of fermentation technologies can guarantee process reproducibility, product safety, and maximal bioactivity.

Keywords: Fermentation Practices; Microbial Entities; Product Development; Raw Milk; Regulations

Introduction

Globally, because of the high cost of health care and medicines, there is an increasing awareness of the importance of a healthy diet and lifestyle. Hence, consumers are increasingly demanding more natural and functional foods, like fermented dairy products. Fermentation, the

second oldest method of food processing, is a metabolic process involving the chemical breakdown of food substances by microorganisms to produce enzymes that initiate chemical changes in an organic substrate, thereby increasing their shelf-life, creating distinct flavors, and enhancing nutritional value (Wilburn & Ryan, 2016). In addition to conventional food fermentation involving

lactic acid, and fungal and alkaline processes (Terefe, 2016), microbial fermentation is instrumental in prolonging shelf life and is vital for human health. Indeed, fermentation technology has significantly improved over the centuries through different approaches, from manufacturing methods, quality control protocols, to overall safety assurance of fermented food product quality/safety (Niyigaba *et al.*, 2025). Such advancements in food fermentation technology are crucial for meeting the rising global demand, which continually requires thorough examination of health/safety implications for the sake of enhanced product integrity (Niyigaba *et al.*, 2025). To ascertain the efficacy of food fermentation technology, very expensive analytical methods are required, such as high-throughput sequencing (HTS), new predictive microbiology models, including novel contamination detection methods like biosensors, polymerase chain reaction (PCR)-based approaches (Ryu *et al.*, 2021), most of which are not easy to acquire by fermentation food industries.

Certain workers have compared milk to a miraculous natural meal, due to its emulsion of fat and its complexity as a material that provides nutrients with functional advantages (Altay, 2017; Gibson & Newsham, 2018). Milk is categorized as a bioactive natural substance (Alonso-Amelot, 2018), offering several health benefits. Raw milk is extremely perishable and might pose health risks to humans based on handling practices and the animal's health; however, thermal preservation is frequently employed (László *et al.*, 2018). Milk fermentation involves significant processes, including proteolytic cleavage and the subsequent release of beneficial compounds (Toldrá *et al.*, 2018). A promising database of bioactive peptides derived from mammalian milk is accessible (Nielsen *et al.*, 2017). The field of zymology, the science of fermentation, has significantly contributed to the improvement of human health, particularly through fermented milk (How *et al.*, 2022). Fermentation of milk involves the necessary metabolic process that utilizes carbohydrate substrates (mostly sugars) in the absence of oxygen (Hansen, 2018) to generate organic acids, gases, or alcohol (Liu *et al.*, 2017). In addition, recognized fermented food products encompass bread, sour milk, cheese, yogurt, and alcoholic beverages such as beer and wine. Furthermore, the properties and physiological benefits of different milks have established them as bioactive natural products (Alonso-Amelot, 2018) engaged in fermentation processes (Toldrá *et al.*, 2018).

Food fermentation processes transpire either naturally or with the aid of a suitable starter culture, in which a viable microbe and its metabolites are preserved (Adewumi, 2019). Yogurt is considered as one of the oldest processed fermented foods, mostly produced using microorganisms or enzymes (Kavitake *et al.*, 2018). It is one of the most

nutritious fermented dairy products due to its beneficial bacteria and bioactive ingredients. Yogurt is a byproduct of lactic acid bacteria (LAB) fermenting milk, possessing antioxidant, antibacterial, and inhibitory properties. Consequently, dairy fermentation processes, particularly those involving milk products, are garnering heightened global research interest due to their contributions of bioactive peptides and functional attributes that promote human health. The health advantages linked to fermentation have intensified the efforts of developed nations to enhance research on probiotics.

Very recent reviews, as summarized in Table 1, have focused on aspects such as specific fermented milk products (Narvhus & Abrahamsen, 2023), bioinformatics devoted to fermented milk products (de Melo Pereira *et al.*, 2022), particular characteristics and microbiology of fermented milk products (Bintsis, & Papademas, 2022), beneficial effects of yoghurts (Hadjimbei, *et al.*, 2022), milk-based dairy beverages, both fermented and nonfermented (Jang, *et al.*, 2024), as well as popular fermented dairy products (Saleem *et al.*, 2024). Given the recent trends that show the importance of fermentation of milk products, there is a great need for continuous updating of the body of knowledge. To supplement existing information, therefore, we hereby attempt a terse review of fermentation intricacies involving dairy milk, seeking to understand the bioactive, health, microbial, and product challenges, drawing from (a) milk—composition and health implications; (b) raw milk pasteurization benefits/concerns; (c) positioning bioactive milk in microbial fermentation; (d) positioning milk protein proteolysis with enzymatic/microbial action; (f) positioning milk product development/preservation with microbial fermentation; (g) microbial entities in milk fermentation and associated/emergent products; (h) microbial influence on fermented milk products' properties; and (i) safety and regulatory pathways enhancing fermented milk products.

Milk—Composition and Health Implications

Milk is the biological fluid produced by the mammary glands of breastfeeding mammals to nourish their offspring and continues to be a fundamental element of human diet. It is the major source of food for newborn mammals and constitutes the primary supply of high-quality protein, calcium, and important micronutrients throughout life (WHO, 2003). Milk has been characterized as a nutritionally dense food with a balanced profile of macronutrients and micronutrients (Fantuz *et al.*, 2016) and as one of the most extensively chemically characterized foods (Renhe *et al.*, 2019). As a result, dietary guidelines in many affluent nations continue to advocate for regular dairy consumption

Table 1. Summary of some very recent reviews conducted on fermented milk products.

No.	Review objective	Scope and key sections covered	Key contribution to fermented milk research	References
1	To describe the origin, evolution, and microbiology of Nordic fermented milk products	Geographical and historical origins; traditional and modern Nordic fermented milks; LAB ecology; metabolism of mesophilic mixed starter cultures; technological developments; health aspects	Provides region-specific insight into mesophilic fermentation systems, starter culture functionality, and links between tradition and modern processing	Narvhus & Abrahamsen (2023)
2	To explore advances in microbial profiling of fermented milk using culture-independent approaches	NGS platforms and workflows; fermented milk microbiomes; bacterial diversity; pathogens and spoilage organisms; bioinformatics tools	Highlights the role of NGS and metagenomics in understanding microbial safety, biodiversity, and quality control in fermented milks	de Melo Pereira <i>et al.</i> (2022)
3	To review fermented milk products (excluding cheese) with emphasis on microbial composition and emerging analytical tools	Types and diversity of fermented milk; market expansion; microbiology of fermented milk products; culture-independent molecular analyses.	Establishes a comprehensive microbiological framework for noncheese fermented milks and identifies research gaps	Bintsis & Papademas (2022)
4	To critically assess the health-promoting effects of yoghurt	Nutritional composition; yoghurt and bone health; gut health; cardiovascular disease; diabetes; immunity; yoghurt as a functional food	Synthesizes clinical and epidemiological evidence linking yoghurt consumption with health outcomes	Hadjimbei <i>et al.</i> (2022)
5	To review milk-based dairy beverages, both fermented and nonfermented, and their functional potential	Probiotics and applications; fermentation vs. nonfermentation; fermented and nonfermented dairy drinks	Broadens scope beyond fermentation and discusses technological and functional roles of dairy beverages	Jang <i>et al.</i> (2024)
6	To evaluate nutritional, microbiological, and therapeutic aspects of major fermented dairy products	Therapeutic effects of yoghurt, kefir, koumiss, and cheese; microbiological and nutritional profiles	Integrates nutritional composition with therapeutic claims across multiple fermented dairy matrices	Saleem <i>et al.</i> (2024)
7	To examine bioactive peptides and functional compounds generated during milk fermentation	Formation of bioactive peptides; LAB proteolysis; health-related bioactivities; technological challenges	Links microbial proteolysis to functional properties and nutraceutical potential of fermented milks	Nongonierma & FitzGerald (2015)
8	To review the role of fermented dairy products in gut microbiota modulation	Fermented milk–microbiota interactions; probiotics; host health outcomes	Strengthens mechanistic understanding of health effects via microbiota modulation	Marco <i>et al.</i> (2017)

of milk as an integral component of a balanced diet, highlighting its significance in skeletal development, metabolic control, and overall nutritional sufficiency (USDA and U.S. Department of Health and Human Services, 2020; Van Horn *et al.*, 2016). The American Heart Association/American College of Cardiology guidelines put forward that adults should take three servings of dairy daily (Van Horn *et al.*, 2016), while the Dietary Guidelines for Americans 2015–2020 for adults recommend the equivalent of three cups a day of fat-free milk (USDA and Department of Health and Human Services (US), 2020).

Nutritional composition of milk with emphasis on cow milk

Cow's milk is the predominant variety of milk globally and serves as the primary component in fermented dairy products. Cow's milk has around 87% water and 13% solids. These solids consist of macronutrients and micronutrients that are readily assimilable by the body. Standard composition values comprise 3.2–3.4% protein, 3.5–4.0% fat, and 4.6–4.9% lactose, along with minerals such as calcium, phosphorus, potassium, magnesium, and zinc (Fox *et al.*, 2017; Walstra *et al.*, 2006).

The protein component of cow's milk is beneficial for human consumption due to its digestibility and balanced amino acid composition. Caseins constitute approximately 80% of all proteins and play a crucial role in facilitating the absorption of calcium and phosphorus into the body via casein micelle structures. Whey proteins (β -lactoglobulin, α -lactalbumin, lactoferrin, and immunoglobulins) support the immune system and generate bioactive peptides during digestion and fermentation (Park and Nam, 2015).

Cow's milk provides a significant source of vitamins, including riboflavin (B2), vitamin B12, niacin, and the fat-soluble vitamins A and D. However, it contains limited iron, and infants may not absorb it effectively, which is particularly crucial for their nutrition. The vitamin and mineral composition of cow's milk is influenced by the lactation period, the animal's genetics, its health status, and its diet (Shan-Shan *et al.*, 2016).

The fat content of milk is crucial for its nutritional value. Cow milk fat contains saturated, monounsaturated, and polyunsaturated fatty acids, along with bioactive lipids, including conjugated linoleic acid (CLA) and short-chain fatty acids. Significantly, modifications in the diet of lactating cows, including pasture feeding or lipid supplementation, can alter the fatty acid composition. This facilitates focused enhancements in nutritional attributes (Kholif *et al.*, 2018).

Several studies indicated a significantly elevated risk of developing iron deficiency anemia in infants who consumed cow's milk compared to those who were given iron-fortified follow-on formula. A separate nonrandomized controlled trial (Griebler *et al.*, 2016) demonstrated reduced hemoglobin levels at 9 and 12 months in infants who were administered cow's milk beginning at 2 months of age, in contrast to those who were given non-iron-fortified formula. The sole study that failed to identify a statistically significant difference in iron status markers compared 21 newborns who consumed cow's milk with 20 infants who ingested low-iron content formula. All infants in that research got iron supplementation of 12 mg/day along with vitamin C supplementation (Griebler *et al.*, 2016). Seven case-control studies, encompassing 2007 cases and 8455 controls, examined the correlation between the age of cow's milk introduction and type 1 diabetes mellitus (T1DM) (Savilahti and Saarinen, 2009). In the revised analysis, all studies except one (Sadauskaite-Kuehne *et al.*, 2004) consistently indicated no variation in the risk for T1DM associated with the early introduction of cow's milk, whether initiated at birth or after 3, 5, 7, or 11 months.

Milk types, alternatives, and comparative health implications

The compositional heterogeneity of mammalian milks presented in Table 2 directly affects nutritional value, allergenicity, fermentability, and product safety. Cow's milk continues to be the primary global substrate for fermented dairy production due to its balanced macronutrient profile, consistent protein structure, and advantageous technological properties (Mituniewicz-Małek *et al.*, 2017). Conversely, high-solid milks like sheep and buffalo milk provide enhanced yields and textural characteristics but necessitate more stringent process control due to their elevated buffering capacity and fat content. Camel and donkey milks offer unique nutritional benefits, such as lower allergenicity and modified whey protein profiles; however, their unusual casein structure and antimicrobial elements may impede standard fermentation, requiring specialized starter cultures and processing conditions (Li *et al.*, 2025; Swelum *et al.*, 2021). Fermented goat milk has exhibited antioxidant, antibacterial, and angiotensin-converting enzyme (ACE)-inhibitory properties (Moreno-Montoro *et al.*, 2017), while functional attributes have also been noted for fermented camel milk, including those fermented with *Lactococcus lactis* KX881782 (Ayyash *et al.*, 2018) and buffalo milk (Basilicata *et al.*, 2018). The compositional variations highlight the necessity of species-specific quality assurance, fermentation design, and safety evaluation in the development or scaling of fermented milk products. Plant-derived milk, known as water-soluble extracts, fundamentally differs in composition from

Table 2. Comparative nutritional composition of selected mammalian milks (approximate values per 100 g).

Milk source	Protein (%)	Fat (%)	Lactose (%)	Casein:Whey ratio	Calcium (mg)	Key compositional and technological notes
Cow	3.2–3.4	3.5–4.0	4.6–4.9	~80:20	~120	Balanced macronutrient profile; high-quality protein; contains β -lactoglobulin; dominant substrate for dairy fermentation (Fantuz et al., 2016; Renhe et al., 2019; Mituniewicz-Matek et al., 2017)
Goat	3.0–3.5	3.8–4.5	4.1–4.7	~70:30	~130	Lower α s1-casein; smaller fat globules; reduced allergenicity compared with cow milk (Clark & Mora Garcia, 2017; Verruck et al., 2019)
Sheep	5.4–6.0	6.0–7.5	4.6–4.9	~80:20	~190	Very high protein and fat; excellent cheese yield; strong buffering capacity (Alavi et al., 2017; Mati et al., 2017)
Camel	3.0–3.5	3.0–3.8	4.5–5.0	~70:30	~110	Lacks β -lactoglobulin; higher whey proteins and lactoferrin; fermentation challenges reported (Al-Ayadhi & Halepoto, 2017; Fugl et al., 2017)
Donkey	1.5–1.8	0.3–1.2	6.0–7.0	~50:50	~70	Close resemblance to human milk; low fat; suitable for CMPA under supervision (Carminati & Tidona, 2017)
Buffalo	3.8–4.5	6.5–8.0	4.7–5.0	~80:20	~180	High fat and calcium; superior texture and yield in fermented dairy (Mati et al., 2017; Basilicata et al., 2018)
Yak	4.5–5.5	5.5–7.0	4.5–5.0	~80:20	~150	Nutrient-dense; adapted to high-altitude environments; limited industrial use (Alavi et al., 2017)
Mare (horse)	2.0–2.5	1.2–2.0	6.2–6.8	~50:50	~90	High lactose; similar to donkey milk; rapid fermentation kinetics (Carminati & Tidona, 2017)
Human	0.9–1.2	3.5–4.5	6.8–7.2	~40:60	~34	Reference standard for infant nutrition (World Health Organization, 2003)

animal-derived milk. Tiger nut milk exhibited phase separation during fermentation; however, the incorporation of dairy proteins mitigated this issue and enhanced the texture (Kizzie-Hayford *et al.*, 2016).

Despite these alternatives, cow’s milk remains the most extensively fermented and technologically versatile dairy substrate. Among fermented milks derived from different animal species, cow’s milk has been reported to exhibit superior processing characteristics, sensory acceptance, and fermentation performance (Mituniewicz-Małek *et al.*, 2017). However, cow milk allergy, primarily associated with β-lactoglobulin, has driven interest in non-cow milk sources such as goat, buffalo, and camel milk (Nuñez, 2016). Goat milk contains lower levels of allergenic proteins and exhibits improved digestibility, making it a suitable alternative for sensitive populations (Clark and Mora García, 2017; Verruck *et al.*, 2019). Although fat globules in cow and goat milk are similar in size, the absence of agglutinin in goat milk reduces fat globule clustering upon cooling, contributing to differences in texture and digestibility (Kalyankar *et al.*, 2016).

Nonbovine milk: nutritional attributes and evidence-based health considerations

In addition to safety issues, there has been increased interest in the nutritional and functional attributes of milk from nonbovine species. Although cow’s milk is the most prevalent variety of milk globally, as indicated in Table 3, milk from camels, goats, buffaloes, and donkeys is garnering increased interest due to its distinctive nutritional and functional attributes. Individuals residing in arid and semi-arid regions, such as Northeast Africa including Ethiopia, consume substantial quantities of camel milk. This is due to camels’ ability to produce milk when cows, sheep, and goats lack sufficient supply (Fugl *et al.*, 2017). Camel’s milk differs from cow’s milk in protein composition, mineral profile, and bioactive protein content, and has primarily been examined in experimental and in vitro models. Furthermore, the caseins in camel milk generate antioxidant peptides that neutralize free radicals, chelate metal ions, and reduce lipid peroxidation (Alhassani, 2024). Numerous studies have demonstrated the antioxidant and antibacterial properties of peptides obtained from camel milk proteins through enzymatic hydrolysis; however, these findings are primarily preclinical and lack evidence of therapeutic efficacy in humans (Agarwal *et al.*, 2003; Al Haj and Al Kanhal, 2010).

Claims of enhanced lactose tolerance may arise from differences in milk protein composition and digestion rates, rather than a true lack of lactose (Khalesi *et al.*, 2017). Donkey’s milk has attracted attention due to its compositional similarity to human milk, particularly its

Table 3. Reported health-related attributes of selected dairy milks and level of evidence.

Milk type	Reported health-related attributes	Level of evidence	References
Camel milk	Investigated for potential neuroprotective and neurological-related effects	Preclinical studies (animal models, observational reports); no confirmed human clinical efficacy	Khattoon & Najam (2017)
Skim camel milk (fermented)	Demonstrates angiotensin-converting enzyme (ACE) inhibitory activity following fermentation	In vitro fermentation and biochemical assays; antihypertensive relevance in humans unproven	Yahya <i>et al.</i> (2017)
Camel milk	Traditionally consumed for gastrointestinal disorders, tuberculosis-associated nutrition, and allergy-related conditions in some regions	Ethnopharmacological use and limited experimental evidence; lacks controlled clinical trials	Mati <i>et al.</i> (2017)
Camel milk	Contains bioactive proteins and peptides associated with wound-healing processes and metabolic modulation	Mechanistic and experimental studies: claims related to autism or diabetes management not clinically substantiated	Alavi <i>et al.</i> (2017); Al-Ayadhi & Halepoto (2017)
Donkey milk	Considered a nutraceutical food with good tolerability and low allergenicity, particularly in cow milk protein allergy	Clinical and nutritional studies under medical supervision; broader health claims remain unverified	Carminati & Tidona (2017)
Cow milk	Provides bioactive growth factors (e.g., transforming growth factor-β) that may support intestinal mucosal development in infants	Physiological and biochemical studies; relevance depends on processing, dose, and developmental stage	Panahipour <i>et al.</i> (2018)

high lactose content, favorable whey-to-casein ratio, and low allergenic potential (Carminati and Tidona, 2017). The appealing attributes of donkey's milk, classified as a nutraceutical product, appear to satisfy the increasing consumer desire for natural and health-enhancing meals (Carminati and Tidona, 2017). Clinical evidence supports its use as a nutritional alternative for infants with cow milk protein allergy under strict medical supervision, while its low-fat content and favorable lipid profile may make it suitable for elderly populations. Despite these attributes, assertions on immune system enhancement, deceleration of aging, or disease prevention lack robust support and require substantial clinical evidence. The health-related features shown in Table 3 should be understood as documented functional relationships rather than therapeutic assertions. For the majority of nonbovine milks, evidence is predominantly sourced from in vitro, animal, or traditional-use scenarios, with minimal corroboration from human intervention trials. Cow's milk is well studied for its physiological significance, especially in baby nutrition. From a quality assurance and safety standpoint, these distinctions are essential to avert overinterpretation of functional potential and to guarantee adherence to regulatory frameworks governing health claims in dairy products.

Health implications of milk consumption

Epidemiological and clinical investigations into the long-term health effects of milk consumption have produced varied findings, reflecting variations in dietary habits, milk processing methods, and individual metabolic reactions (Givens, 2012). Increasing evidence supports the association between milk consumption and positive health outcomes, including enhanced bone mineral density, reduced hypertension risk, and improved metabolic health. Fermented dairy products appear to augment these benefits by generating bioactive peptides and modifying gut microbiota.

Researchers have extensively studied cow's milk for both children and adults. Consistent data indicate that the premature introduction of unmodified cow's milk during infancy (before to 12 months) increases the risk of iron deficiency anemia due to its low iron content, reduced iron bioavailability, and the potential for intestinal blood loss (Griebler *et al.*, 2016). Controlled investigations, both randomized and nonrandomized, indicate that babies receiving cow's milk exhibit decreased hemoglobin concentrations compared to those provided with iron-fortified formula, unless iron supplementation is implemented. Conversely, concerns regarding a correlation between early exposure to cow's milk and T1DM have not been consistently substantiated. Meta-analyses and case-control studies have shown no consistent

association between the age of cow milk introduction and the incidence of T1DM, after controlling for confounding variables (Savilahti and Saarinen, 2009). Post-infancy, fermented cow milk products appear to offer enhanced benefits due to their improved digestibility, production of bioactive peptides, and modulation of gut microbiota. Cow milk distinguishes itself from fermented milks of other species due to its superior technological properties and sensory appeal, which contributes to its dominance in global dairy fermentation (Mituniewicz-Małek *et al.*, 2017). Cow milk allergy, primarily attributed to β -lactoglobulin, has spurred research in alternative milks, including goat and camel milk, which often exhibit less allergenicity (Nuñez, 2016; Clark and Mora García, 2017; Verruck *et al.*, 2019).

Because milk is such an important part of the human diet, a full assessment of its health impacts needs to combine large-scale epidemiological data with mechanistic studies that look at digestion, metabolism, and microbial interactions (Zhang *et al.*, 2021; Papatheodorou, 2019). These kinds of integrated approaches are necessary to improve dietary recommendations and make milk and milk-based products more nutritious and useful.

Raw Milk Pasteurization Benefits/Concerns

Pasteurization of milk remains an important step prior to fermentation, as the ingestion of raw (unpasteurized) milk poses significant public health hazards, unlike pasteurized milk (Anadón *et al.*, 2017). Pasteurization, which is the use of controlled heat to kill harmful germs, has been one of the best ways to keep food safe in the last hundred years (FDA, 2024a). Because so many people started using pasteurized milk, milk-borne diseases including tuberculosis, brucellosis, and Q fever, which were historically spread through contaminated dairy products, dropped sharply (CDC, 2023). Some people still promote drinking raw milk, saying it has better nutrients, more probiotics, or better digestion, even though this is not true. There is no controlled scientific evidence to back up these claims. Numerous surveillance investigations have shown that raw milk can contain harmful bacteria like *Salmonella enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Brucella spp.*, even when it is made on a clean farm (Oliver *et al.*, 2009; Claeys *et al.*, 2013). CDC's data show that raw milk and its products are more likely to bring about foodborne disease outbreaks compared to pasteurized dairy products, and those who get sick as a result are more likely to be hospitalized (Weinstein, 2025). Infants, pregnant women, elderly adults, and people with weakened immune systems are all more likely to have serious problems such as hemolytic uremic syndrome, septicemia, miscarriage, and newborn infection (FDA, 2024b).

Some consumers believe that pasteurization of raw milk tampers with its unique aromas, textures, colors, and flavors (Licitra *et al.*, 2019). However, there is no proof that pasteurization greatly lowers the nutritious value of milk. Heat treatment causes only small losses of those vitamins that are susceptible to heat, like vitamin C and folate, which are not important for nutrition in milk. Also, protein stability and sensory qualities of milk, such as flavor, are affected by heating. Macronutrients, calcium, and fat-soluble vitamins are mostly unaffected (Walstra, *et al.*, 2006). Furthermore, pasteurization does not hinder lactose digestion, since milk lacks endogenous lactase in physiologically significant quantities, and lactose intolerance is not associated with heat treatment of milk (Lucey, 2016). Claims that raw milk improves immune function or inhibits allergies and asthma are equally unsubstantiated. Epidemiological studies have indicated a reduced prevalence of allergies in children reared on farms; however, this “farm effect” is ascribed to increased environmental microbial exposure rather than the ingestion of raw milk itself (von Mutius and Vercelli, 2010). Alternative methods to treat milk for pathogen inactivation are under research, including the use of high pressure to destroy pathogens and decrease spoilage microorganisms (Bucci *et al.*, 2018). Other treatments like thermo-sensation, microwave heating, and ohmic heating can also be used to treat the product to inactivate pathogenic microorganisms and reduce spoilage microorganisms. Consumer opinion on the microbiological safety of raw milk is very divided because raw milk is believed to be natural with some desirable characteristics (Tavares & Malcata, 2019). The complex nature of dairy (supply) chain further elevates the ease by which microbial contamination can occur. Important microorganisms relevant to milk spoilage during handling would be key indicators about its hygienic status, like psychrotrophs, mesophilic aerobes/coliforms, among others, which are largely pathogenic (Perin *et al.*, 2019). Some consumers prefer fermented milk given its health benefits (such as probiotic property) and sensory properties, like its characteristic taste/flavor.

Overall, scientists agree that pasteurization is good for public health and has very few negative effects on nutrition. Raw milk does not offer distinct health benefits and has a considerably elevated risk of foodborne illness. So, to keep dairy safe, current public health guidance emphasizes continued adherence to pasteurization requirements, robust regulatory oversight, and evidence-based education about dairy safety based on facts, especially for people who are at higher risk.

Positioning Bioactive Milk in Microbial Fermentation

Microbial fermentation is the main process that changes the bioactive profile of fermented milk products by

making both good and bad metabolites with different starting cultures having very different metabolic capacities, as shown in Figure 1. This directly affects the production of bioactive compounds and, as a result, the choice of fermentation cultures. Bioactive peptides are of special interest among these metabolites since their physiological importance has been shown (Meleti *et al.*, 2025). These peptides are low-molecular-weight protein fragments, usually made up of 2 to 20 amino acid residues. They come from parent milk proteins during fermentation when microbes break them down (Agyei *et al.*, 2017; Martínez-Villaluenga *et al.*, 2016). Conversely, certain fermentation-derived substances, such as biogenic amines, are typically regarded as undesirable owing to their potential toxicity, highlighting the necessity for meticulous selection of starting cultures.

Caseins and whey proteins in milk are two of the most important sources of bioactive peptides (Hafeez *et al.*, 2014; Hazlett *et al.*, 2019). Proteolytic LAB break down these proteins during fermentation, generating peptides that have antibacterial, antihypertensive, antioxidant, and immunomodulatory effects (Martínez-Villaluenga *et al.*, 2016). Fermented dairy products are considered one of the primary dietary sources of bioactive peptides, with peptide production being highly contingent on the proteolytic activity of the utilized starter cultures (Elkhtab *et al.*, 2017). Profiling studies of fermented milk products, including kefir and yogurt, have identified various peptides that demonstrate ACE inhibitory and antibacterial properties, thereby affirming the functional significance of fermentation-derived peptides (Ebner *et al.*, 2015; Huang *et al.*, 2018).

The capacity of LAB to produce bioactive peptides is mostly ascribed to their well-defined proteolytic systems (Shuang *et al.*, 2026). Members of the order *Lactobacillales*, including *L. lactis*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, possess intricate enzymatic systems comprising cell-envelope proteinases and intracellular peptidases, including endopeptidases, aminopeptidases, dipeptidases, and tripeptidases (Akbarian *et al.*, 2022; Griffiths and Tellez, 2013; John *et al.*, 2006; Song *et al.*, 2017). These proteolytic systems break down milk proteins into tiny peptides and free amino acids in steps, which helps bacteria proliferate and make bioactive peptides. These microbes are important for technology as well as biology since they help fermented dairy products acquire texture, flavor, and overall quality (Akbarian *et al.*, 2022). Various approaches have been investigated to augment bioactive peptide synthesis during milk fermentation. Using highly proteolytic starter cultures, mixed or sequential fermentations, and combining fermentation with controlled enzymatic hydrolysis have all been found to boost peptide yield and

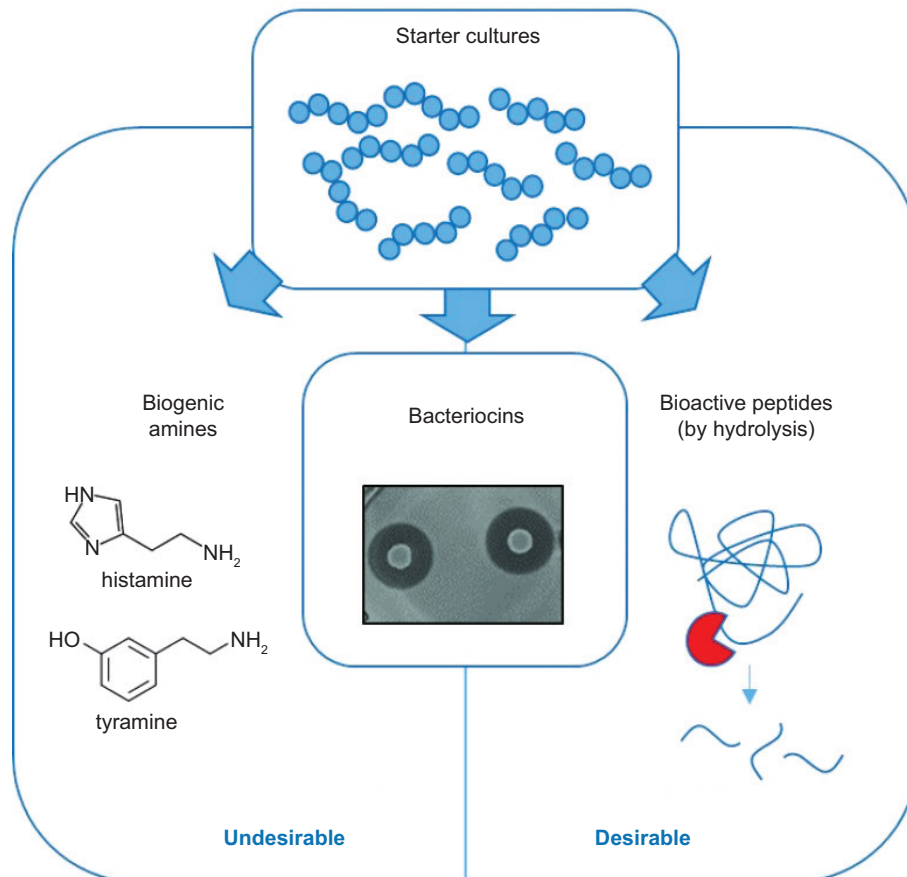


Figure 1. Desirable and undesirable bioactive metabolites produced during fermentation (Source: Hill *et al.*, 2017a; an open access article with Creative Commons Attribution License).

functional activity (Akbarian *et al.*, 2022; Chai *et al.*, 2020). For instance, using exogenous proteases like trypsin before fermentation has been shown to help release phosphopeptide-rich fractions from caseins. This includes calcium-binding casein phosphopeptides, which are only made in small amounts during fermentation alone (Lorenzen *et al.*, 2005). Fermented milk products made with mixed LAB cultures have also shown more ACE-inhibitory activity than single-strain fermentations (Chen *et al.*, 2007).

Bacteriocin synthesis by LAB is frequently considered a beneficial probiotic characteristic because of its inhibitory effects on spoilage and pathogenic microbes; nevertheless, it may also present technological obstacles. Bacteriocins can stop starter strains from growing together, which could change the speed of fermentation and the uniformity of the final product, especially in mixed-culture systems (Hill *et al.*, 2017a). So, while choosing starting cultures for industrial fermentation operations, it is important to carefully consider how

much bacteriocin they produce. The increasing interest in functional foods has solidified bioactive milk peptides as attractive nutraceutical components (Singh *et al.*, 2018). These peptides can be added directly to functional dairy products or made during fermentation to make the products work better (Arihara *et al.*, 2017; Barberis *et al.*, 2018), and they have been attributed with benefits such as antioxidative, ACE-inhibitory, antithrombotic, and antimicrobial activities, and modifying of immune response (Giacometti & Buretić-Tomljanović, 2017). Bioactive peptides have been shown to help probiotics stay alive and stable in fermented milk products, which improves both the microbiological and functional quality of the products (Kronic *et al.*, 2018). Their antihypertensive potential is particularly significant, given the global rise in the prevalence of hypertension. Fermentation with specific LAB strains has demonstrated an increase in ACE-inhibitory activity in milk, suggesting the targeted release of antihypertensive peptides during the fermentation process (Elkhtab *et al.*, 2017; Ozturkoglu-Budak, 2017).

In general, bioactive peptides are an important link between microbial fermentation, product quality, and health-oriented functionality in fermented dairy systems. Starter culture choice, fermentation conditions, and the availability of protein substrates all have a big effect on how they form. It is still important to include proteolytic performance, bioactive potential, and technical compatibility in the design of starter cultures for the creation of safe, consistent, and functionally improved fermented milk products.

Positioning Milk Protein Proteolysis with Enzymatic/Microbial Action

Proteolysis is a crucial biochemical process that facilitates milk fermentation, product maturation, and the formation of bioactive peptides (Aouadhi, 2025). In raw milk, proteolytic activity arises from both intrinsic milk enzymes and enzymes produced by contaminating or intentionally introducing microorganisms. Plasmin, the most extensively researched and functionally significant endogenous milk protease, is an alkaline serine protease characterized by its stability at elevated temperatures and optimal activity on casein substrates, particularly β -casein and α s2-casein (Ismail & Nielsen,

2010). Its efficacy is diminished with κ -casein (Vaghela et al., 2018). It remains effective post-pasteurization and facilitates the breakdown of proteins in milk during storage and processing.

Microbial proteases, together with native enzymes, are very important for breaking down milk proteins during fermentation. These enzymes may come from microbes that were accidentally introduced during handling or from starter and adjunct cultures that were added on purpose. *Bacillus*, *Pseudomonas*, *Micrococcus*, *Clostridium*, and *Escherichia* are all types of proteolytic bacteria that are often found in milk. *Aspergillus* spp. are filamentous fungi that are often used in food biotechnology because they make a lot of extracellular proteases (Kavitake et al., 2018; Khedkar et al., 2016;). LAB in fermented dairy systems mostly use cell-envelope proteinases and internal peptidases instead of a lot of external proteolysis. This lets them break down proteins in a way that is safe and good for the product.

Figure 2 shows that milk protein proteolysis during fermentation can be thought of as having two parts: enzymatic and microbial. These two parts work at the same time and are often reliant on each other. Enzymatic proteolysis entails the hydrolysis of peptide bonds by

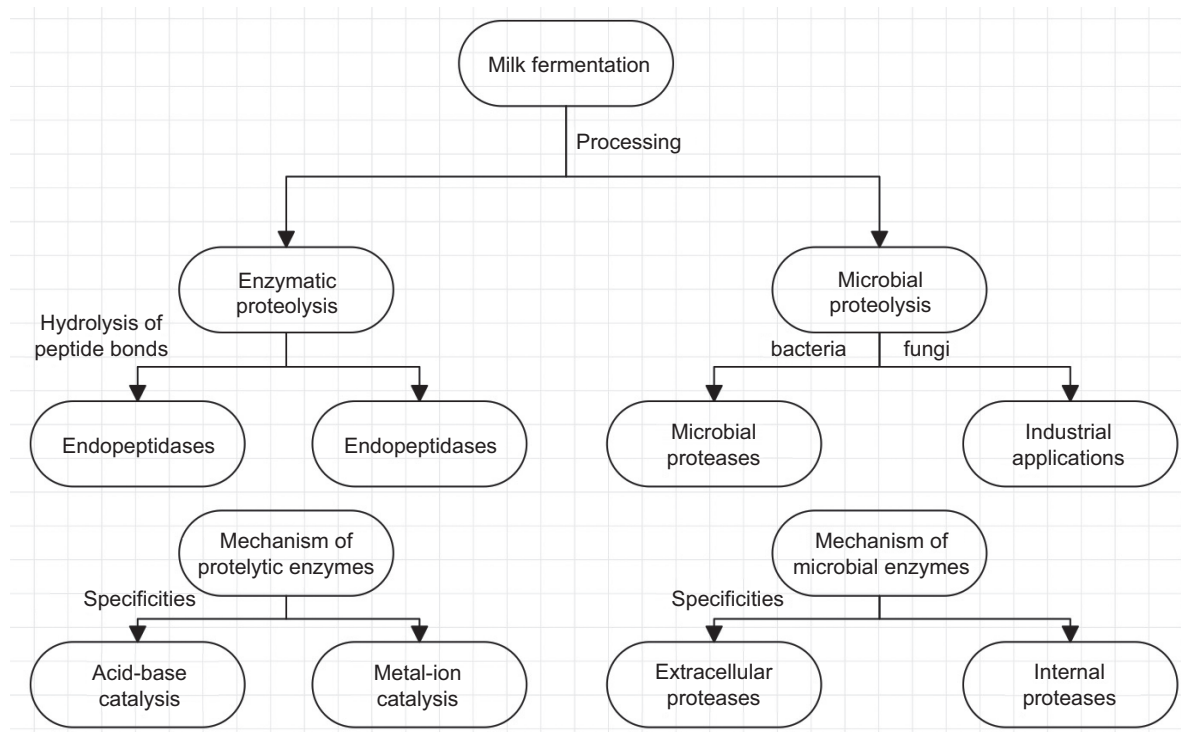


Figure 2. Breakdown of protein proteolysis, applicable to milk fermentation, clearly divided into enzymatic and microbial aspects. Under each, there is the mechanism and their respective specificities.

proteases, such as endopeptidases and exopeptidases, utilizing specific catalytic methods that include acid–base catalysis and, occasionally, metal ions. Microbial proteolysis, in contrast, includes both the breakdown of proteins outside of cells and the processing of peptides inside cells once they have taken up a substrate. This depends on the type of organism and the conditions of fermentation (Kavitake *et al.*, 2018; Song *et al.*, 2023). Proteolytic reactions typically occur via three conserved mechanistic phases at the molecular level. The first step is the creation of the enzyme–substrate complex, which is also called the Michaelis complex. In this step, the protease attaches to the target peptide sequence in a way that is not covalent. This phase decides how specific the substrate is and how well the enzyme works as a catalyst. It can either move on to catalysis or go back to free enzyme and substrate (Tripathi and Nair, 2013). Peptide bond cleavage is the second step. It is a hydrolytic reaction that needs water and is sped up by proteases that activate the scissile bond. This stage is very important for breaking down proteins, making them mature, and making sure they are of good quality in both biological and food systems (Klein *et al.*, 2018). In the third stage, either a water molecule or an amino acid residue, usually serine, cysteine, or threonine, attacks the carbonyl carbon of the peptide bond in the enzyme active site. The kind of protease determines which amino acid residue is used (Koutinas, 2017; Niyigaba *et al.*, 2025; Vaghela *et al.*, 2018). This attack creates a temporary tetrahedral intermediate that then breaks down, breaking bonds and releasing peptide fragments while also making the free enzyme again (Saha *et al.*, 2020). The effectiveness of this mechanism is determined by the geometry of the active site, the catalytic residues, and the stability of reaction intermediates.

To manage proteolysis during fermentation, one needs to know how endogenous milk enzymes and microbial proteases work together. Moderate proteolysis is good for making flavors, changing textures, and making bioactive peptides, but too much or unregulated protease activity can make things bitter, cause structural problems, and lower the quality of the product. So, choosing the right starting cultures, keeping microbial contamination under control, and managing the conditions of processing are still very important for getting balanced proteolysis in fermented milk products.

Positioning Milk Product Development/ Preservation with Microbial Fermentation

Using LAB to ferment milk is one of the best and oldest ways to keep it fresh. It also helps manufacture a larger range of dairy products and improve their flavors. Most industrial and artisanal methods heat milk before

fermentation to kill germs and make sure the process is the same every time. People across the world enjoy fermented milk products because they taste wonderful, make you feel good, and are highly excellent for you. The creation of bioactive compounds during fermentation, many of which are associated with functional and physiological effects, as seen in Table 4, elucidates their widespread acceptance. Researchers have extensively examined the health advantages of fermented milk products, particularly regarding probiotics, dietary support, growth modulation, and their role as transporters for useful chemicals. Yogurt and other fermented dairy products have been shown to be effective vehicles for probiotic delivery, enhancing gut microbiota balance and host health (Utz *et al.*, 2017; Feng *et al.*, 2022; Fazilah *et al.*, 2018). Researchers have also looked into using fermented dairy matrices as the basis for dietary supplements and functional foods, such as adding fruits or other bioactive compounds to improve the formulation (How *et al.*, 2022; Lopes de Oliveira *et al.*, 2022; Mohanty *et al.*, 2016b). People’s enjoyment of fermented milk products is greatly affected by their sensory characteristics. Taste is always rated as the most significant attribute, followed by texture and mouthfeel (Pan *et al.*, 2014). Fermentation not only keeps milk fresh, but it also makes it healthier by making it simpler to digest, reducing the amounts of antinutritional agents, and making some nutrients more available. Food is safer and healthier for the environment when it has greater sensory qualities, a larger range of products, and less energy is needed to prepare it (Bevilacqua *et al.*, 2016; Ohshima and Giri, 2014). Fermented milk products have unique tastes because they contain amino acids, peptides, and other low-molecular-weight chemicals that are formed when proteins and carbohydrates are broken down during fermentation (Zhao *et al.*, 2016). People used spontaneous fermentation to keep milk fresh when there was no way to cool it down. This suggests that the major objective of fermentation was to keep food from becoming stale, and the second goal was to make it taste better and provide health advantages (Gagnaire and Jan, 2017). In the end, the quality and effects of fermented dairy products depend on how flavor development, nutritional enhancement, and health-related functioning work together (Al-Dhaheri *et al.*, 2017).

Nonconventional starter cultures and alternative fermentation methods have attracted attention due to their ability to operate under varying pH and temperature conditions. For example, researchers have investigated using Kombucha-based consortia as extra cultures in milk fermentation to add new flavors and functions (Kanurić *et al.*, 2018). From a technical point of view, it is thought to be even difficult to ferment milk that is not from cows, given the culture’s capability within the fermentation system. Camel milk also possesses antimicrobial properties and reduced proteolytic activity, which can also slow down the fermentation process.

Table 4. Reported health-related effects of fermented milk products.

Category	Reported health-related effects	References
Probiotic effects of fermented milk	Fermented milk products such as yogurt and kefir contain probiotic microorganisms that may modulate gut microbiota and are associated with reduced risk of certain cancers	Utz et al. (2017)
Yogurt and healthy aging	Regular yogurt consumption has been associated with markers of healthy aging and longevity	Fazilah et al. (2018)
Dietary management and metabolic health	Fermented milk products, particularly yogurt and kefir, have been investigated for their potential roles in managing obesity, type 2 diabetes, and cardiovascular risk factors	Mohanty et al. (2016a); Nongonierma & FitzGerald (2015); Pothuraju et al. (2018); Jangra et al. (2019); Miraghajani et al. (2017)
Anti-obesity effects	Kefir and yogurt consumption has been associated with reduced adiposity and improved lipid metabolism in experimental and human studies	Mohanty et al. (2016a); Pothuraju et al. (2018); Jangra et al. (2019)
Age-related health support	Fermented milks containing <i>Lactobacillus acidophilus</i> and <i>Bifidobacterium</i> spp. may alleviate age-related pathologies and support healthy aging	Kapila et al. (2017); Wichansawakun & Buttar (2019); Turkmen et al. (2019)
Growth and developmental relevance	Cow milk-based yogurt contains bioactive growth factors, including transforming growth factor- β (TGF- β), which may contribute to intestinal mucosal homeostasis	Panahipour et al. (2018)
Prebiotic-enriched fermented milk	Fruit by-products rich in dietary fiber and polyphenols have been incorporated into fermented milk to enhance prebiotic potential, reduce inflammation, and mitigate gut dysbiosis	Lopes de Oliveira et al. (2022)
Vehicle for bioactive delivery	Fermented milk matrices have been explored as carriers for oral vaccine delivery because of their protective and immunomodulatory properties	How et al. (2022)
Inflammatory bowel disease (IBD)	Probiotic fermented milk may help attenuate inflammatory responses and restore gut microbial balance in individuals with IBD	Feng et al. (2022)
Neuroactive and biofunctional properties	Fermented milk containing γ -aminobutyric acid (GABA) has been associated with sedative, antihypertensive, anti-diabetic, antioxidant, and immunomodulatory effects; evidence is largely mechanistic and experimental	Ramos & Poveda (2022); Beltrán-Barrientos et al. (2018); Mati et al. (2017); Mora et al. (2019)
Immunomodulatory effects	Fermented milk products may exert immunotherapeutic effects by modulating immune responses and host-microbe interactions	Brennmoehl et al. (2018); Wang et al. (2015)
Cholesterol metabolism	Regular consumption of probiotic fermented milk has been associated with reductions in blood cholesterol levels	Vieira et al. (2017)

Adding protein substrates like casein to camel milk systems has been shown to improve fermentation (Berhe *et al.*, 2018). Using some LAB strains, like *Lactobacillus para-casei* and *Lactobacillus plantarum*, to ferment sheep milk has been proven to improve viscosity and sensory qualities. This shows how important it is to choose the right strain for product optimization (Ramos and Poveda, 2022). Also, LAB are still the most common bacteria employed to ferment milk. However, not all dairy products, like some cheeses, need active fermentation. Fermented foods are the main source for individuals to get live beneficial bacteria in their diets. The functional potential of LAB has resulted in the continuous isolation and characterization of novel strains possessing advantageous technical and health-related attributes (Khedkar *et al.*, 2016; Teneva-Angelova *et al.*, 2018). LAB may do a lot of different things with their metabolism, such as breaking down proteins, fats, and sugars. These activities are important for generating tastes, textures, and bioactive substances. When milk ferments, LAB can create exopolysaccharides (EPS), which are usually high-molecular-weight heteropolysaccharides that can be either slimy or ropy. These compounds are particularly important for the texture, mouthfeel, and stability of fermented dairy products. People typically employ *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to create yogurt. They interact together to generate lactic acid, thicken protein, and modify the texture (Ni *et al.*, 2018). Some strains of *S. thermophilus* are known to create EPS, which is what

gives some kinds of yogurt their unique texture (Khanal and Lucey, 2018). The progressive reduction in pH during fermentation, which happens because lactic acid builds up, is an important step in creating yogurt. This process can be done in a set, stirred, or pre-acidified fashion, depending on the product's specifications.

Figure 3 illustrates that the fermentation of milk is regulated by a complex interplay among the composition of raw milk, the emergent microbiota, processing conditions, and selected starter cultures. This interaction dictates the spectrum of fermentation products produced (Yang *et al.*, 2025). Fermented dairy products, primarily derived from domesticated animals, encompass a wide variety of foods, with yogurt and cheese being the most widely consumed globally (Koutinas, 2017). The increasing popularity of yogurt is attributed to its distinctive flavor, substantial nutritional value, and the presence of bioactive and antibacterial compounds beneficial to health (Fazilah *et al.*, 2018; Marco *et al.*, 2017). Functional dairy products can be developed through the intentional selection of LAB strains that enhance both nutritional value and biological activity (de Moreno de LeBlanc *et al.*, 2018). Fermented dairy products with *Lactobacillus* spp. are associated with antioxidative and anti-inflammatory properties, underscoring their importance in health-focused dietary strategies (Iwasa & Aoi, 2017). Fermented dairy products are important sources of bioavailable vitamins and minerals, including

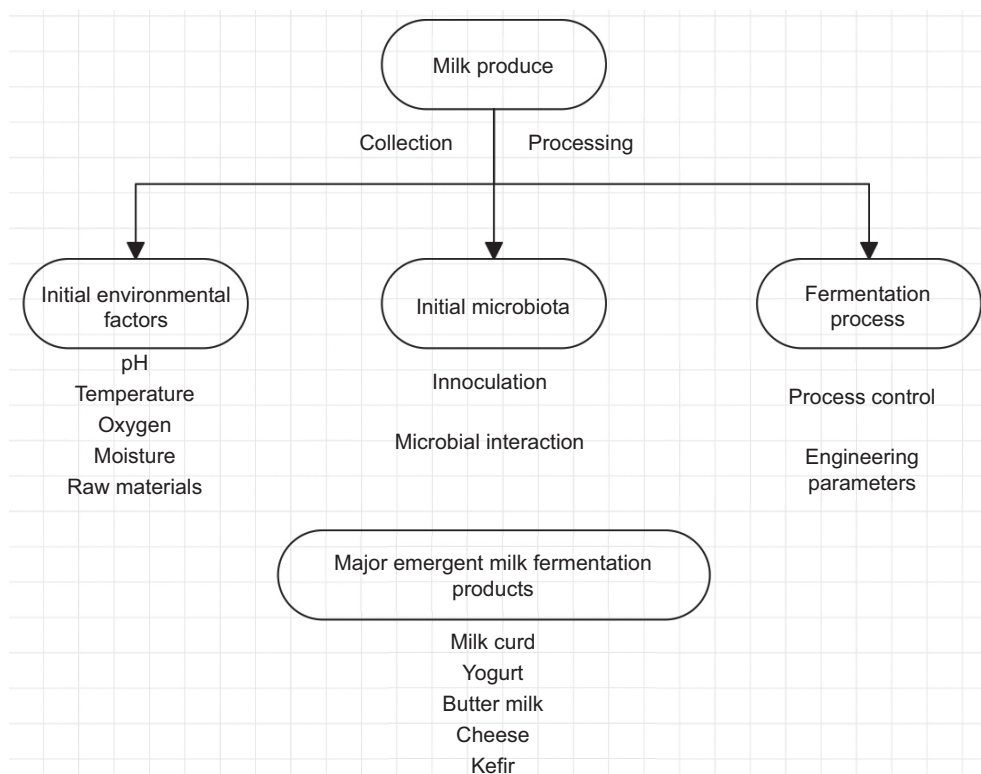


Figure 3. Major factors/key players in the milk fermentation process, and emergent products.

calcium, phosphorus, magnesium, zinc, iodine, and B vitamins, as fermentation often enhances nutrient bioavailability relative to unfermented milk (Fernandez *et al.*, 2016; Garcia-Burgos *et al.*, 2020). Yogurt is widely regarded as a nutritionally balanced fermented dairy product, primarily due to its live microbial content (probiotics) and the formation of bioactive compounds during fermentation, which confer advantages over milk (Behare *et al.*, 2016; Fernandez *et al.*, 2016). Interest in the health benefits of yogurt has existed since the early 1900s but has intensified in recent decades due to advancements in studies on gut bacteria (Hill *et al.*, 2017b). Yeasts coexist with LAB in traditional fermented dairy products. This enhances the gastronomic intricacy and the diversity of microorganisms. Commercial probiotics predominantly consist of bacterial strains such as lactobacilli and bifidobacteria; however, certain yeasts, like *Saccharomyces boulardii*, are also recognized for their health benefits (Bourrie *et al.*, 2016).

In a nutshell, fermented milk products are associated with a broad spectrum of health-associated functionalities, primarily driven by probiotic activity, bioactive peptides, and fermentation-derived metabolites. While many reported benefits—particularly those related to metabolic health, immune modulation, and gut function—are supported by experimental and observational studies, the strength of evidence varies across outcomes and product types. Consequently, these effects should be interpreted as functional associations rather than substantiated therapeutic claims, emphasizing the need for well-designed human intervention trials to confirm causality and define effective consumption levels.

Microbial Entities in Milk Fermentation and Associated/Emergent Products

Microorganisms that help milk ferment are a big part of the probiotic and functional benefits of fermented dairy products. Yogurt, because of regulated fermentation, demonstrates antioxidant properties and can be categorized as probiotic food if it contains live microorganisms with proven health advantages. LAB are the most common starter cultures for dairy fermentation because they are dependable, work well with technology, and have been shown to have probiotic potential (Chan *et al.*, 2018). Consequently, numerous fermented milk products with probiotic or nutraceutical properties have been created, such as acidophilus milk fermented with *Lactobacillus acidophilus* (Kongo and Malcata, 2015).

Experimental research demonstrates that probiotic fermented milk can alter the makeup of gut microbiota and enhance intestinal health. For instance, the intake of fermented milk with a standard starter culture enhanced by several probiotic strains demonstrated a partial

restoration of gut microbiota equilibrium in a rat model of dextran sulfate sodium-induced inflammatory bowel illness (Feng *et al.*, 2022). Probiotic foods are defined as meals that contain live microbes that, when taken in the right amounts, improve the health of the person who eats them (Wang *et al.*, 2015). These microorganisms are mainly LAB, especially species from the *Lactobacillus* and *Bifidobacterium* genera. Because of the increasing incidence of gastrointestinal inflammation and associated illnesses, probiotic LAB utilized in milk fermentation have garnered interest for their immunomodulatory and anti-inflammatory properties (Brenmoehl *et al.*, 2018).

Microbial selection is a crucial factor influencing the functional characteristics of fermented milk. The selection of fermenting strains for yogurt is based on the desired nutritional or physiological effect. For example, chosen strains of *S. thermophilus* with better folic acid biosynthetic capacity have been used to make yogurt with more folate (Meucci *et al.*, 2018). But it's crucial to remember that not all yogurts are probiotic meals. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are traditional yogurt starter cultures. They are chosen mostly for their technological performance, and they don't naturally give yogurt probiotic status until they are mixed with other probiotic strains. One well-known benefit of yogurt over unfermented milk is that it helps those who can't digest lactose better and helps people who can't absorb minerals better (Freitas, 2017).

The bacteria that are most employed to ferment milk are *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Their combined metabolism causes acidification, protein coagulation, and texture development (Li *et al.*, 2022). In addition to producing acid, some types of bacteria can be chosen to make fermented milk products thicker, more stable, and better tasting (Wang *et al.*, 2022). *Lactiplantibacillus plantarum*, a well-known probiotic species, has been demonstrated to improve both the functional properties and the shelf life of fermented milk, which helps keep the product fresh and the probiotics alive (Huang *et al.*, 2022; Wu *et al.*, 2017). Researchers have also investigated using other ingredients to improve the effectiveness of probiotics and the speed of fermentation. For instance, adding cupuaçu (*Theobroma grandiflorum*) pulp to goat milk yogurt has been reported to speed up fermentation because it has a lot of fermentable carbohydrates like sucrose, glucose, and fructose (Costa *et al.*, 2016). The addition of polyphenol-rich grape pomace extract to milk before fermentation sped up the process of acidification and made it easier for probiotic bacteria like *S. thermophilus* and *L. acidophilus* to stay alive while being stored in the fridge (de Souza de Azevedo *et al.*, 2018). These results show that plant-based chemicals could improve the effectiveness of probiotic dairy systems. Cheese consumption, a type of fermented dairy product, has been linked to several

health benefits, such as increased mass, lower blood pressure, changes in low-density lipoprotein cholesterol, and better dental health. However, these effects depend on how much cheese is eaten and what kind it is (Tunick and Van Hekken, 2015). Alongside bacterial probiotics, certain yeasts have also exhibited probiotic effectiveness. *Saccharomyces boulardii* has demonstrated efficacy in alleviating symptoms of *Clostridium difficile*–associated diarrhea by diminishing intestinal inflammation and modifying immunological responses, hence suggesting its application as an adjuvant in diarrhea care (García-Burgos *et al.*, 2020).

Putting together probiotics with prebiotics has resulted in the creation of symbiotic products, wherein prebiotic substrates specifically enhance microbial proliferation and metabolic functions within the gastrointestinal system. Prebiotic supplementation has demonstrated the ability to augment the antibacterial activity and longevity of probiotic strains, hence enhancing their functional efficiency (Pranckutė *et al.*, 2016). Symbiosis is a new way to improve the health of both the host and the probiotics (Pranckutė *et al.*, 2014). The International Scientific

Association for Probiotics and Prebiotics defines fermented meals and beverages as those created via regulated microbial proliferation and enzymatic transformation of food constituents (Hill *et al.*, 2017a). Milk fermentation, necessitating specialized microbial consortia, yields a diverse array of traditional and industrial products, including yogurt, kefir, kumis, curd, cultured milk, sour milk, buttermilk, acidophilus milk, and various fermented cheeses (de Oliveira, 2014). Traditional fermented milk products are globally widespread and deeply embedded in local food cultures, reflecting adaptation to regional climates, livestock species, and preservation needs. Many fermented milk products are region-specific, reflecting local microbial resources, cultural practices, and raw material availability, as summarized in Table 5 (Altay, 2017; Alu'datt *et al.*, 2016; Bokulich *et al.*, 2015; Danova *et al.*, 2017; Guo *et al.*, 2018; Macuamule *et al.*, 2016; Mudgal and Prajapati, 2017; O'Callaghan *et al.*, 2019; Owusu-Kwarteng *et al.*, 2017; Özer and Kirmaci, 2014). These products are typically produced through spontaneous or back-slopping fermentation, resulting in highly diverse microbial consortia dominated by LAB and, in some cases, yeasts. Increasing molecular

Table 5. Selected examples of traditional fermented milk products by geographic origin.

S/N	Product	Location	References
1	Nunu	West Africa (Nigeria, Ghana)	Owusu-Kwarteng <i>et al.</i> (2017)
2	Omashikwa	Namibia	Macuamule <i>et al.</i> (2016)
3	Omaere	Namibia	Macuamule <i>et al.</i> (2016)
4	Kulenaoto, Kwerionie, Ehekapmkaika, Chekapmkaika	Uganda	Macuamule <i>et al.</i> (2016)
5	Chambiko	Malawi	Macuamule <i>et al.</i> (2016)
6	Masse	Mozambique	Macuamule <i>et al.</i> (2016)
7	Kefir	Eastern Europe and Central Asia (Caucasus region)	Özer & Kirmaci (2014); Gul <i>et al.</i> (2015)
8	Matsoni	Georgia, Armenia	Bokulich <i>et al.</i> (2015)
9	Koumiss (Airag)	Mongolia and Central Asia	Guo <i>et al.</i> (2018)
10	Katak	Bulgaria	Danova <i>et al.</i> (2017)
11	Dahi	Indian subcontinent	Mudgal & Prajapati (2017)
12	Ayran	Turkey	Altay (2017)
13	Jameed	Eastern Mediterranean (Jordan, Iraq)	Alu'datt <i>et al.</i> (2016)
14	Tarhana (milk–cereal fermented product)	Middle East, Balkans, Central Asia	O'Callaghan <i>et al.</i> (2019)
15	Leben (Lben)	North Africa (Morocco, Algeria, Tunisia)	Benkerroum (2013)
16	Raabadi/Rob	Sudan and East Africa	Dirar (1993)
17	Suusac	Kenya, Somalia	Farah <i>et al.</i> (2016)
18	Ititu	Ethiopia	Ashenafi (2006)
19	Shubat	Kazakhstan, Turkmenistan	Wernery (2006)
20	Filmjölök	Scandinavia (Sweden, Finland)	Narvhus & Abrahamsen (2023)
21	Skyr	Iceland	Narvhus & Abrahamsen (2023)
22	Amasi	Southern Africa (South Africa, Zimbabwe)	Beukes <i>et al.</i> (2001)
23	Chhurpi (soft)	Himalayan regions (Nepal, Bhutan)	Tamang (2009)

and metagenomic studies demonstrate that such indigenous fermented milks are reservoirs of unique microbial biodiversity, which may underpin their reported nutritional, functional, and sensory attributes. Understanding these traditional systems is critical not only for cultural preservation but also for bioprospecting novel starter cultures, improving food safety, and developing region-specific functional dairy products with reproducible quality.

Microbial Influence on Fermented Milk Products' Properties

A comprehensive comprehension of microbial impacts on milk characteristics during fermentation initiates with the recognized function of LAB, their primary physiological classifications, and the settings that facilitate their optimal development. Wouters *et al.* (2002) did some of the first work to categorize the LAB employed in dairy fermentations into two main groups based on their ideal temperatures. Mesophilic LAB grow best between 20°C and 30°C, while thermophilic LAB grow best between 30°C and 45°C. This classification elucidates the geographical distribution of traditional fermented milk products, with thermophilic LAB prevalent in fermented dairy foods from subtropical and tropical regions, whereas mesophilic LAB are more typical of products from Western and Northern Europe.

The activity of starter cultures and their metabolic processes are what really determine the outcome of fermentation. No matter what kind of fermented milk product it is, mesophilic *Lactococcus* species have been utilized as starter cultures for a long time. These cultures are very important in determining the final sensory profile of fermented milk, especially its flavor. This is because they use enzymes to convert amino acids into volatile and nonvolatile flavor molecules (Wouters *et al.*, 2002). However, thermophilic LAB are still the most well-known starter cultures for making fermented milk. Most of them come from spontaneous acidification processes that are caused by native microflora (Hafeez *et al.*, 2014; Kavitate *et al.*, 2018). These starting organisms help make flavored molecules that are unique to each type of food, as well as other vital metabolites for technology, like EPS and folic acid, by breaking down proteins. Yeasts are found in certain fermented dairy systems, although their role is typically regarded as secondary, despite the common linkage of fermented milk products with naturally occurring yeast-containing microbiota (Wouters *et al.*, 2002).

Fermentation of milk is a process that is driven by microbes that changes the milk's properties in a big way, affecting its digestibility, flavor, aroma, and texture (Fernández *et al.*, 2015; Fusco *et al.*, 2020). LAB change lactose into lactic acid, which lowers the pH of milk over time and causes

the proteins in the milk to clump together. This acidity not only makes gels form, but it also makes the conditions right for the creation of the unique smells and tastes that come from fermented milk products. At the same time, microbial enzyme systems help break down proteins and carbs, making nutrients more available and easier to digest (Fernández *et al.*, 2015; Fusco *et al.*, 2020). The main changes that happen during milk fermentation include a drop in pH during coagulation, the creation of aroma and flavor compounds, better digestibility, and a wider range of microbes involved in the process (Ortiz-Rivera *et al.*, 2017; Verruck *et al.*, 2019). Lactic acid buildup decreases the pH of milk and makes casein clump together, which makes the fermented matrix thicker and changes its structure. These alterations markedly affect the texture and mouthfeel of fermented dairy products (Bourrie *et al.*, 2016; Turkmen *et al.*, 2019; Wilburn and Ryan, 2016).

Another important result of microbial activity during milk fermentation is the formation of flavor and aroma. LAB and other bacteria make a wide range of metabolites, such as organic acids, alcohols, esters, and other volatile chemicals. These metabolites give fermented milk its unique sensory properties (Costa *et al.*, 2016; de Moreno de LeBlanc *et al.*, 2018). Fermentation also makes food easier to digest by breaking down macronutrients like proteins, lipids, and lactose into free fatty acids, peptides, and bioactive compounds that the body can absorb more easily (Kok and Hutkins, 2018; Koutinas, 2017; Marco *et al.*, 2017). The microbial community that ferments milk can be very different from one place to another, and the conditions in which the milk is processed and the type of milk used can have a big effect on this. This variety of microbes is what gives fermented milk products their unique tastes and functional properties. This is why there are so many kinds of products, from cheese and kefir to yogurt. The unique tastes, textures, and nutritional qualities of diverse fermented dairy products are ultimately determined by the interaction between certain microbial consortia and milk substrates.

Safety and Regulatory Pathways Enhancing Fermented Milk Products

Fermentation was first used to make food and drinks last longer and safer, easier to digest, and better tasting. Fermented items, especially fermented dairy foods, have become more important in the last few decades because they have been shown to be good for health (Şanlıer *et al.*, 2017). Yogurt, sour milk, and cheese are examples of fermented dairy products that have a lot of bioactive peptides that come from nature. Milk is a rich and easy-to-get source of bioactive peptides that have antibacterial, antihypertensive, antioxidant, antithrombotic, and immunomodulatory effects (Park and Nam,

2015). Lactoferrin is a milk protein that is known for its ability to kill and stop bacteria from growing, mostly via binding to iron (Niaz *et al.*, 2019). Other proteins and peptides found in milk, like α -lactalbumin and certain peptide fragments like CAMP211–225, have been shown to fight pathogenic microorganisms like *Escherichia coli*, *Yersinia enterocolitica*, and *Staphylococcus aureus* that are resistant to antibiotics (Brück, 2005; Nibbering *et al.*, 2001; Wang *et al.*, 2020).

Fermented dairy products have garnered significant epidemiological attention because of their correlation with diminished risks of obesity and chronic conditions, including metabolic, cardiovascular, immune-related, and neurocognitive problems (Kok and Hutkins, 2018). The consumption of yogurt has been associated with the modification of immune function, specifically through the lowering of inflammatory biomarkers and the regulation of both humoral and cellular immunological responses (Asemi *et al.*, 2011; Meyer *et al.*, 2007). These advantages are increasingly attributed, at least in part, to fermentation-induced modifications in gut microbiota composition and activity (Kok and Hutkins, 2018). In addition to the effects of fermentation, milk and dairy products have been linked to a wide range of physiological functions, such as anti-carcinogenic, anti-inflammatory, antioxidant, anti-adipogenic, antihypertensive, anti-hyperglycemic, and bone-protective properties (Cadogan *et al.*, 1997; Da Silva *et al.*, 2015; He *et al.*, 2011; Milard *et al.*, 2019; O'Connor *et al.*, 2019; Parodi, 1997; Sultan *et al.*, 2018). Because milk is such an important part of human diet, a full assessment of its health effects must look at a wide range of illness endpoints (Papatheodorou, 2019; Zhang *et al.*, 2021).

Improvements in food processing technologies have made fermented dairy products even more useful and safe. High hydrostatic pressure processing (HHP), sometimes called ultra-high-pressure processing, has become a promising nonthermal method that can improve microbiological safety while keeping the taste and nutritional value (Abera, 2019; Khaliq *et al.*, 2021; Tonello-Samson *et al.*, 2020). When the pressure is higher than 600 MPa, heating caused by compression can raise the temperature of a product enough to kill germs or pasteurize it, depending on the conditions of the process (Ozaybi, 2024). HPP is primarily used for treating foods that are prepackaged in high-barrier multilayer flexible packaging and offers an effective alternative for microbial inactivation while preserving the sensory and nutritional quality of the product by utilizing pressure-induced heat (Júnior *et al.*, 2023). HPP has clear benefits, such as being good for prepackaged goods and causing little heat damage. However, there are still problems, such as adiabatic temperature increases and changes to milk proteins and mineral equilibria caused by pressure (Balasubramaniam *et al.*, 2015; Woldemariam and Emire, 2019).

Regulatory control is very important for making sure that fermented foods are safe. Food safety rules are different in different parts of the world, but they always stress that producers are responsible for making sure that food cultures are used safely (Puvaca and Vapa, 2024). Generally, Southeast Asia faces various challenges in fermentation, notably enhancing safety and quality without compromising the characteristic flavors and authenticity of the products (Wache *et al.*, 2018). Regulatory frameworks in places such as Japan, South Korea, and the European Union have emerged to accommodate both conventional and new fermentation processes, including those employing genetically modified microorganisms (Mukherjee *et al.*, 2022; Tanaka *et al.*, 2023). The European Food Safety Authority (EFSA) oversees strictly by enforcing microbiological criteria, process hygiene standards, and HACCP-based systems in the European Union. The EFSA also focuses on risk assessment and finding new safety problems (Niyigaba *et al.*, 2025).

As fermentation moves beyond typical dairy systems to encompass plant-based substrates and new microbial communities, rules and regulations are changing to protect public health (Tan *et al.*, 2024). The U.S. Food and Drug Administration has given lactoferrin the “Generally Recognized as Safe” (GRAS) classification, while the European Commission has approved bovine lactoferrin for use in certain food categories with certain limits (Franco *et al.*, 2018). Adding it to baby formulas, fermented milks, yogurts, drinks, and functional coatings shows how fermentation science, functional nutrition, and regulatory oversight are all coming together in new ways in the dairy industry (Garcia-Montoya *et al.*, 2012; Quinteri *et al.*, 2013; Yilmaz and Tosun, 2013).

Current Limitations and Unresolved Challenges

Notwithstanding significant progress in starter culture biotechnology, high-throughput analytics, and process control, numerous obstacles persist that hinder the reproducibility and application of dairy milk fermentation systems. At the process level, variability in raw milk composition between batches, ambiguous consortia in conventional fermentations, and strain-specific variances in LAB can result in variable metabolite profiles and sensory consequences, even under ostensibly standardized conditions (Smid & Lacroix, 2013). Recent mechanistic reviews of LAB starter interactions in fermented milk elucidate how symbiotic networks among *S. thermophilus*, *L. delbrueckii subsp. bulgaricus*, *L. lactis*, and adjunct strains exhibit nonlinear responses to pH, redox state, nutrient availability, and quorum-sensing signals, emphasizing the necessity for more quantitative,

systems-level models to forecast fermentation trajectories (Garcia-Gonzalez *et al.*, 2023).

The standardization of starting cultures continues to be a significant difficulty. Commercial consortia are often categorized at the species level, although recent genomic and phenotypic data indicate that health-related properties, including exopolysaccharide production, bioactive peptide release, bacteriocin synthesis, and stress tolerance, can be markedly strain-specific (O'Callaghan & van Sinderen, 2016; Walsh *et al.*, 2017). Recent evaluations of dairy LAB-derived bioactives and antimicrobial agents highlight that these functional outcomes are contingent upon the specific combination of strains, the history of culture maintenance, and the settings employed during fermentation (Silva *et al.*, 2018; Leroy & De Vuyst, 2022). A critical demand exists for globally accessible, well-defined culture collections, regular whole-genome sequencing, and standardized phenotypic assays to provide cross-study comparisons of starter performance and safety characteristics (Shuang *et al.*, 2026).

A subsequent constraint pertains to the therapeutic application of bioactive peptides and probiotic functions revealed *in vitro* or through animal models. Revised inventories of milk-derived peptides and dairy fermentate components now enumerate thousands of sequences exhibiting antihypertensive, antioxidant, immunomodulatory, or metabolic activities (Kashung & Karuthapandian, 2025); however, only a limited subset has undergone thorough evaluation in meticulously designed human intervention trials with definitive primary outcomes (Niyigaba *et al.*, 2025). Recent *in vivo* syntheses of dairy bacteria and fermented dairy products suggest beneficial correlations with enhanced gastrointestinal function, specific cardiometabolic indicators, and immune responses; however, they also reveal variability among strains, dosages, matrices, and endpoints, along with frequent underpowering for significant clinical outcomes (Marco *et al.*, 2021; Dimidi *et al.*, 2022). The existing gaps constrain the robustness of causal inferences derived from the current evidence and necessitate more focused, sufficiently powered randomized controlled studies that associate specific strains or peptide fractions with defined clinical outcomes (Marco *et al.*, 2021).

Ultimately, regulatory harmonization has lagged behind advancements in dairy fermentation and functional dairy products. Frameworks like the European Food Safety Authority's Qualified Presumption of Safety list and the United States Food and Drug Administration's Generally Recognized as Safe designations offer systematic approaches for evaluating the safety of traditional starter species and dairy-derived bioactives; however, there is

less consensus on the assessment of complex multistrain probiotics, postbiotics, and peptide-rich fermentates utilized in functional foods and nutraceuticals (Hill *et al.*, 2014). Variations in allowable health claims, evidence standards, and labeling regulations among jurisdictions hinder the global marketing of fermented dairy products with claimed health advantages (Niyigaba *et al.*, 2025). Resolving these outstanding problems necessitates collaborative endeavors spanning microbiology, process engineering, clinical research, and regulatory science to transition from descriptive characterization to predictive, reproducible, and clinically validated dairy fermentation systems.

Conclusion and Future Outlooks

This terse review has critically analyzed the intricacies of dairy milk fermentation and provided useful insights regarding microbial dynamics, bioactive component production, health ramifications, and product-related obstacles. Evidence suggests that microbial fermentation of milk has significant potential to provide additional health advantages, notwithstanding the impact of commercial limitations and regulatory frameworks. Most times, the outcomes of fermentation processes depend on very complex interactions between microbial consortia and the milk matrix, which in the end would determine the degree of functionality of the emergent product as well as its stability. Besides, fermented milk products would deliver several health benefits, such as antibacterial, antioxidant, and ACE-inhibitory properties when consumed. Clinical and experimental research substantiate their functions as immunomodulatory, anti-carcinogenic, hypocholesterolemic, antioxidant, and hypotensive agents.

Nonetheless, there is a need for enhanced regulation of fermentation technologies to guarantee process reproducibility, product safety, and maximal bioactivity. Enhancing the selection of starter cultures, aided by molecular, omics-based, *in silico* methods, alongside strain efficacy, viability, and functional predictability should be an area for future research. Also, the molecular identification/bioavailability evaluation of bioactive components in fermented milk should be useful in facilitating customized adjustments of organoleptic and nutritional characteristics, which future workers can explore especially to address specific consumer health requirements. Further, increased focus is warranted on indigenous fermented dairy products, probiotic effectiveness, molecular connections between microbial metabolism and host health, which should come from additional evidenced-based information such as clinical trials involving animals and human.

Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that no AI-assisted tools were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

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Author Contributions

All authors contributed equally to this article.

Conflicts of Interest

The authors declare no conflict of interest.

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