The Evolution of Fermented Milks

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The manufacture of fermented milk products has a long history, and these products were initially produced either from spontaneous fermentation or using a batch of previously produced product, that is, back-slopping. Milk of different mammal species has traditionally been used for the manufacture of fermented milk products. Cow's milk is the basis for most dairy fermented products around the world. Milk from other mammals, including sheep, goat, camel, mare, buffalo, and yak may have been historically more important and remain so in certain regions. The milks from different species have differences in chemical composition and in certain, vital for the fermentation, components. The diversity of fermented milk products is further influenced by the wide variety of manufacturing practices. A great number of fermented dairy products have been traditionally produced worldwide, and many of them are still produced either following the same traditional process or manufactured industrially, using standardized processes under controlled conditions with specified starter cultures.

Keywords: fermented milk products ; fermented dairy products

1. The Expansion of Fermented Milk Products

The remarkable expansion of fermented milk products started in the early 20th century, after Metchnikoff's proposal [1] that the apparent longevity of the hill tribesmen of Bulgaria was a direct result of their life-long consumption of yogurt inspired an interest in the nutritional characteristics of the product that has never abated [2]. Human gut microbiome research has revealed the link between the gut microbiome and different aspects of human health and diseases, and this finding has necessitated studies on fermented foods and their roles in enhancing the microbiome [3]. Functional and therapeutic yogurts and fermented milks have reached the markets, since the successful commercial probiotic milk beverage, Yakult, which was launched in 1935 in Japan [4].

Yogurt is one of the most popular fermented milk products worldwide. Originating from the Balkans and the Middle East, it has become a major component of the human diet worldwide [5]. Although homemade yogurt is still produced using the "back-slopping method" worldwide, the growing global attention and the increasing demand, led to the production of yogurt on an industrial scale, with full control of the production procedures and the use of heat-treated milks and starter cultures [6]. Yogurt has a viscosity and a distinctive acidic, sharp flavor [7][8]. Yogurt is produced by the symbiotic growth of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, both present naturally in milk or added as starter culture at 40–45 °C. *Str. thermophilus* grows faster than *Lb. delbrueckii* subsp. *bulgaricus* and then ferments the lactose in the presence of dissolved oxygen and releases more lactic acid, formic acid, and CO_2 from urea, compounds that encourage the growth of *Lb. delbrueckii* subsp. *bulgaricus*. In the presence of formic acid, *Lb. delbrueckii* subsp. *bulgaricus* stimulates *Str. thermophilus* by releasing essential or stimulatory amino acids through its proteolytic system [7][9].

During the first years of industrial production, yogurt had limited acceptability in North Americans and European consumers, since natural yogurt can taste extremely acidic to Western palates, and it was not until the various forms of sweetened and fruit-flavored yogurt went on sale that the market for yogurt really expanded. With innovation in packaging and materials, the concept of stirred fruit yogurt as a pleasant and nutritious snack was the main reason for the forthcoming expansion [2]. Yogurt is manufactured today following a very similar procedure as thousands of years ago and remains the most important fermented milk product. It is presented to the consumer in either a gel form (set type, which is incubated and cooled in the final package) or as a viscous fluid (stirred type, which is incubated in the tank with the coagulum to be broken before cooling and packaging) and more locally as a concentrated product. The drinking type yogurt, which is similar to stirred yogurt, has the coagulum broken before cooling, but with more severe agitation. Concentrated yogurt is inoculated and fermented just like stirred yogurt, with the difference that after the breaking of the coagulum, the yogurt is concentrated by boiling off some of the water. These concentrated yogurts are often called strained yogurts or strained fermented milks because of the straining of the whey from the coagulum [10][11]. A special concentrated yogurt is Greek yogurt or Greek-style or Stragisto, which has been strained in a special cloth (tsantila) and thus whey is removed giving a product with 21–23% total solids [11], while Labneh is a famous fermented product from Middle East, strained from the traditional yogurt in a special cloth bag for 10–14 h to remove the whey, and some salt can be added to improve the shelf life [12]. Frozen-type yogurt is inoculated and incubated in the same process as stirred yogurt, but the cooling is carried out by pumping through a whipper/chiller/freezer in a process similar to the production of ice cream [7]. From the variety of traditional yogurts, and with increasing success in the global dairy market, novel yogurts and yogurt-like products have entered the markets, for example, frozen yogurts, liquid yogurts, fruit-yogurts, strained yogurts, probiotic yogurts, bio-yogurts, therapeutic yogurts; these have acquired enormous market success.

Kefir, is a viscous, acidic and mildly alcoholic fermented milk, with a refreshing taste, originated from the Caucasus region of Asia; it is produced by natural fermentation or from the inoculation of the kefir grains, that is, back-slopping, in milk [8][12][13][14]. Kefir grains are composed of an insoluble protein and polysaccharide matrix, gelatinous, and yellowish and vary in size from 0.3 to 3.5 cm in diameter [15]. Kefir has, during the last 10 years, presented an enormous expansion in the global markets. Koumis, Kumis, Kumys, or Qumys is another naturally fermented milk product from the Caucasian area, India, Mongolia, and the Middle East. Similar products such as Chigee and Airag are produced in Mongolia and northwestern China, [16][17]. The process is similar to that of Kefir and produces a gray-colored liquid milk, lightly carbonated with a sharp alcoholic and acidic taste [18][19].

Traditional dairy fermentations can be performed either by natural, that is, spontaneous fermentation, or by back-slopping. Both types of fermentation of milk are mediated by LAB, which consume lactose and produce lactic acid [20][21]. The most common dairy LAB include species from four main genera: *Lactococcus, Lactobacillus*, *Leuconostoc*, and *Pediococcus*. In addition to forming lactic acid, these bacteria also modify other constituents of milk resulting in increased bioavailability of nutrients and enhanced quality [7][22]. In addition, LAB and their metabolic products, mainly bacteriocins, inhibit spoilage and pathogenic microorganisms [23][24][25][26].

The main disadvantage of the back-slopping method is that the final product may not always be equally stable in taste and quality, as well as pose a high risk of loss of starter culture activity, for example, by bacteriophages and, as a result, the loss of product [16]. The spontaneous fermentation of milk has been largely displaced by the addition of well-characterized and well-defined starter cultures [27][28][29][30]. Dairy cultures consist of selected and well-defined strains of LAB species that are produced in concentrated and stable forms [20]. Their wide availability, ease of use, and consistent properties have made them common even in developing countries [31]. Starter cultures used in milk fermentation include *Lactococcus lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Leuconostoc* spp., and *Str. ther-mophilus*; the main function is the acidification of the medium. However, additional functions are performed such as contribution to the development of texture, flavor, and biochemical changes [29][30][32][33].

The majority of commercial fermented milks in the markets are manufactured using a mixture of non-traditional and traditional starter cultures. Presently, a great variety of fermented dairy products, based on traditional ones, are manufactured worldwide under controlled conditions with specified starter cultures. Even before sustainability was recognized as an issue in agriculture, fermented dairy products were associated with many of the key elements of sustainable food production [31]. They were manufactured in dairy farms which were self-sufficient with fermented milks being produced and consumed on the location of the farm. Certain characteristics in the production of fermented milks, such as the optimal use of natural and human resources, respect for biodiversity and ecosystems, being environmentally sound, and economically fair and viable, providing the consumer with nutritionally adequate, safe, healthy, and affordable food, meet the requirements of the definition of food sustainability [34]. Raw materials, that is milk from different species, used to make fermented dairy products, were traditionally obtained locally and provided consumers with safe, nutritious, and affordable foods. Fermentation is usually conducted under mild conditions, consuming little energy relative to other forms of food processing, and little waste or by-products are generated [31][34]. In addition, safety is also a global sustainability issue, and for certain autochthonous LAB with antimicrobial properties, the use of protective cultures or the addition of herbs may provide biopreservation to ensure food safety and extended shelf-life at very low cost [35].

Fermented milks are recognized as one of the most popular fermented products due to their extended shelf life and characteristic organoleptic properties, as well as, for their health benefits [36][37][38][39][40][41][42][43]. The functions that have been associated with fermented milk products are schematically shown in **Figure 1**.

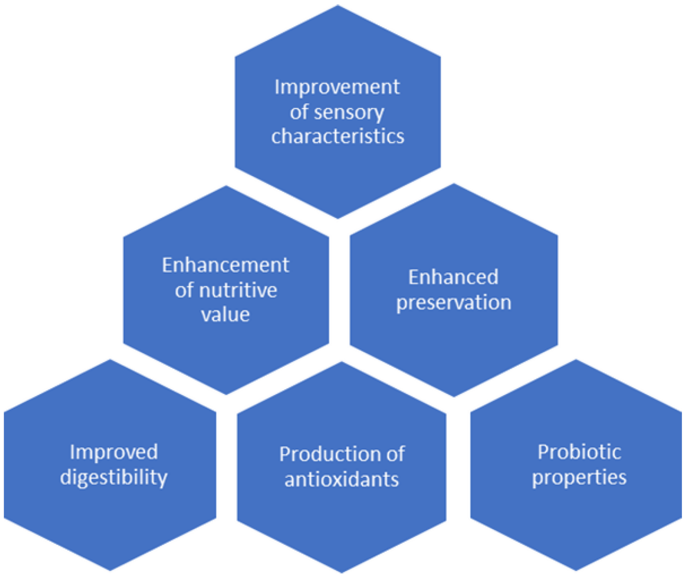

Figure 1. Functions associated with fermented milk products.

The enhancement of the nutritive value is related to the production of certain vitamins from LAB, as well as the increased essential amino acids in fermented milks [18][44]. Studies on Trachana fermentation showed a significant increase in riboflavin, niacin, pantothenic acid, ascorbic acid, and folic acid contents of the product [45][46]. The inclusion of red pepper as an ingredient in Tarhana increased the α-tocopherol and carotenoid contents and antioxidant activity and improved the fatty acid profile [47][48]. The improvement of sensory characteristics is related to the production of flavor compounds, for example, diacetyl, from LAB has been reported to modify certain milk components resulting in increased bioavailability of nutrients and enhanced quality [22]. The enhanced preservation is achieved with the production of antimicrobial compounds that is organic acids, hydrogen peroxide, diacetyl, and bacteriocins with antagonistic microbiological properties to suppress the growth of undesirable microbiota [49][50][51][52][53].

The improved digestibility of milk by the fermentation process is one of the main health benefits of fermented dairy products; this was probably the main reason for the very early acceptance of fermented milks from lactose-intolerant groups. Lactose intolerance is associated with diarrhea and flatulence induced by lactose metabolites. Because of these, it is nutritionally beneficial to remove lactose; for example, by converting it to lactic acid when fermenting milk, and removing the fraction containing lactose when making fermented dairy products [54]. As a result of the fermentation process conducted using LAB and yeasts, only a little concentration of lactose remains in the final product. Perna et al. showed that the lactose content gradually decreased during storage in yogurt and probiotic yogurt from donkey milk [55].

The production of bioactive compounds, namely conjugated linoleic acid, an anticarcinogenic agent, by *Lb. acidophilus* has been studied [56]. Manzo et al. studied the effects of probiotics and prebiotics, that is, *Bifidobacterium animalis* subsp. *lactis* on conjugated linoleic acid and determined the contents of 10 commercial fermented milk products; they reported that the highest content was observed in fermented milk containing only *Str. thermophilus* and *Lb. delbrueckii subsp. bulgaricus* [57]. Recently, the optimization of certain techniques for maximizing the production of conjugated linoleic acid by *Bifidobacterium animalis* in fermented milk samples was studied [58].

Viili and similar products are made in Sweden, Norway, Denmark, and Iceland [7] and many of these products share a thick and sticky consistency due to the development of extracellular polysaccharide (EPS)-producing strains of *Lactococcus* spp. [59][60]. Besides the technological significance, the health benefits of EPS have also been reported, as they act as nutritional components for colonocytes, liver, and muscle cells, and modulate the host immune system [61]. Antioxidant activity is one of the key functions of the peptides taken from milk proteins and this activity is attributed to the peptides from the proteolysis of casein and to the whey proteins. In addition, *Lb. acidophilus* has been reported to increase the antioxidant activity of yogurt made by autochthonous and commercial starter culture [62].

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit to the host” and fermented dairy products are probably the most important food probiotics category; probiotic fermented milks have been extensively studied [38][39][40][41][63][64][65][66][67][68][69][70]. Fermented dairy products are generally beneficial in the treatment and prevention of gastrointestinal disease, considering that different LAB strains show different efficacy across these diseases. Limdi et al. reviewed the therapeutic role of probiotics in gastroenterology and concluded that probiotics appear to have a potential role in the prevention and treatment of various gastrointestinal illnesses, such as irritable bowel syndrome, but it is likely that benefits are species and strains specific [71].

Hypercholesterolemia occurs when there is an elevated level of total cholesterol in the bloodstream and the ingestion of probiotic LAB might be a more natural way to decrease serum cholesterol in humans. Several animal studies have shown that the administration of fermented milks is effective in lowering blood cholesterol levels, although studies in human subjects have shown conflicting results [64].

Observational studies in humans support the importance of the intestinal microbiota in immune development and have found a relationship between probiotics and the development of allergic disease, for example, the treatment of childhood eczema [67].

Clinical evidence has shown that *Lactobacillus rhamnosus* GG can prevent and contribute to the recovery from rotavirus-associated diarrhea in children [72]. Although the mechanism for this protective effect is not clear, it has been shown that *Lb. rhamnosus* GG is able to bind to the mucosal surface of the intestine [73], possibly protecting against intestinal pathogens and associated infections through immunomodulation [74].

Ingestion of probiotic yogurt has been reported to stimulate cytokine production in blood cells and enhance the activities of macrophages [75]. Yakult is a Japanese commercial probiotic milk product that has several health-promoting benefits such as modulation of the immune system, maintenance of gut flora, regulation of bowel habits, alleviation of constipation, and curing of gastrointestinal infections [76]. The modulation of the gut microbiota by the administration of *Lactobacillus kefiranofaciens* has been studied in mice [77].

Fermented milks were suggested to have a beneficial effect on cardiometabolic health and especially on type 2 diabetes [78]. Ayyash et al. compared camel to bovine fermented milk and reported in vitro anticancer, antihypertensive, antidiabetic, and antioxidant activities of camel fermented milk [79]. The anti-obesity effect of yogurt fermented by *Lb. plantarum* Q180 in diet-induced obese rats has been studied [80].

During the last 10 years, the functional properties of Kefir were extensively studied [13][14][81][82][83][84], as well as those of yogurt [85].

2. Microbiology of Fermented Milk Products

Another point that has driven the evolution of fermented milk products is the application of culture-independent methods for the identification of microbiota. The microbiota of fermented milk products has been extensively studied using classical microbiology, that is, using culture-dependent methods and phenotypic identification methods. These methods have given significant insights into specific isolates and microbial populations, but the culture media used may not be sufficiently selective for monitoring population dynamics and may fail to recover unculturable bacteria, resulting in an underestimation of microbial diversity [86]. Direct DNA extraction from samples of fermented foods commonly referred to as culture-independent methods is commonly used in food microbiology to profile both cultivable and uncultivable microbial populations from fermented foods [87]. Both culturable and unculturable microbes from any fermented dairy product may be identified using culture-dependent and culture-independent methods; the latter methods had an impact on revealing inter- and intra-species diversity within a particular genus or among genera [88][89]. The most popular culture-independent technique being used in the isolation of microorganisms from fermented foods is a PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis to profile bacterial populations [87] and yeast populations in fermented foods [89][90]. Wolfe and Dutton reviewed the microbial communities of fermented foods and concluded that these communities offer a wide range of paradigms for community formation and provide opportunities to understand how to better design synthetic microbial communities for medicine, industry, and agriculture [91]. The omics approaches have contributed to understanding how these microbes affect the organoleptic properties of fermented dairy products, such as the metabolome and volatilome, and other functional and quality attributes.

Liu et al., 2012 analyzed the bacterial composition of Kurut in Tibet using culture-independent methods, a bacterial 16S rRNA gene clone library containing 460 clones was constructed and the bacterial diversity in Kurut was systematically studied; the authors reported some novel sequences of unknown bacteria [92]. To provide a better understanding of microbial ecology in Kurut, the application of the traditional culture method combined with molecular biology technology would be very useful, and future studies on microbial diversity in traditional fermented dairy products should employ both culture-dependent and culture-independent methods. Watanabe et al. used culture- and molecular biology-based methods to identify 367 LAB strains and 152 yeast strains isolated from Airag and Tarag samples in Mongolia. *Lacticaseibacillus casei*, *Lb. delbrueckii* spp. *bulgaricus*, *Limosilactobacillus fermentum*, *Lb. kefiranofaciens*, *Lactiplantibacillus plantarum*, *Lc. lactis* spp. *cremoris*, and *Str. thermophilus* were the most commonly isolated species [93]. Kochetkova et al. studied the microbiome of more than 50 fermented dairy products from Russia, using culture-independent next-generation sequencing (NGS), and reported that the microbiomes of the same dairy products from different regions were similar in dominant microorganisms and varied mainly in the minor parts of the community [94].

The use of culture-independent methodology has revealed the complex microbiota of kefir grains, which includes a mixture of bacteria such as *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*, and *Lc. lactis* subsp. *cremoris, Lb. kefiranofaciens, Lentilactobacillus kefiri, Lentilactobacillus parakefiri, Lb. helveticus, Lb. delbrueckii, Lcb. casei, Levilactobacillus brevis, Lacticaseibacillus paracasei, Lpb. plantarum* and *Leuc. mesenteroides*, *Lactobacillus helveticus*, *Leuconostoc citreum*, *Leuconostoc gelidum*, *Leuconostoc kimchi, Acetobacter pasteurianus*, and *Acetobacter lovaniensis* [9][95][96][97][98][99][100], and yeasts such as *Kl. marxianus*, *Saccharomyces cerevisiae, Torulopsis kefir, Torulaspora delbrueckii, Candida kefir, Saccharomyces unisporus, Pichia fermentans, Yarrowia lipolytica, Debaryomyces* spp., *Galactomyces* spp., *Issatchenkia* spp., *Kazachstania* spp., *Kluyveromyces* spp., *Pichia* spp., *Saccharomyces* spp., *Wickerhamomyces* spp. and *Yarrowia* spp. [9][101][102]. Kesmen and Kacmaz, using culture-dependent methods identified in Kefir grains and Kefir *Lc. lactis*, *Leuc. mesenteroides* and *Lb. kefiri* as prevalent species, while using PCR-DGGE as a culture-independent method identified *Lb. kefiranofaciens* and *Lc. lactis* as prevalent [103]. Interestingly, Kefir can be made from different milks and the microbiota has been reported to be different from that found in fermented milk as the complex symbiotic interactions between the microbes in the grains differ from those in the milk [104]. The amplicon-based analysis of Kefir from different countries, both in Europe and America, revealed the absence of any clear clustering of the associated microbiomes on the basis of geography; and it was apparent that the populations present in the fermented milk (Kefir) were more homogeneous than the corresponding grains (Kefir grains) from which they were produced [104]. It should be noted that the sequencing data confirmed the change in the species composition and quantitative ratios of the Kefir microbiota with the predominance of lactococci in the final product. Differences between the microbiota of Kefir milk and Kefir grains have also been confirmed by other studies using culture-dependent and culture-independent approaches [105]. Newer identification techniques, like whole metagenome shotgun sequencing, provide more detailed information about the overall microbial structure, in particular for species of low abundance. These methods were able to provide a broader view of the microbial composition and population dynamics of Kefir [106][107]. Recently, Alraddadi et al., 2023 studied the microbial communities of Kefir grains, and Kefir was evaluated over time using high-throughput amplicon sequencing. It was found that *Lb. kefiranofaciens* and *Lentilactobacillus kefiri* consistently dominated Kefir grains, whereas *Lc. lactis* dominated Kefir [107]. Many other bacteria and yeasts were detected that comprised the minor population of Kefir grains and Kefir. The community composition in the kefir was more variable than in the Kefir grains with the relative abundance of both *Lb. kefiranofaciens* and *Lc. lactis* changing over time. The fungal communities of Kefir grains were dominated by *Kazachstania turicensis* and *T. delbruekii*, although the ratio between the two varied significantly. These findings suggest that the microbial communities in Kefir grains change over time, highlighting the need for further studies investigating the effect these changes have on the production of flavor and aroma compounds in Kefir [107].

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