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Review paper

Running title: Fermented and non-fermented milk with probiotics

Overview of Dairy-based Products with Probiotics: Fermented or Non-fermented Milk Drink

Abstract

Probiotic products have long been recognized for their health benefits. Additionally, milk has held a longstanding reputation as a dairy product that offers high-quality proteins and essential micronutrients. As awareness of the impact of food on health grows, interest in functional products such as probiotic dairy products are on the rise. Fermentation, a time-honored technique used to enhance nutritional value and food preservation, has been used for centuries to increase nutritional value and is one of the oldest food processing methods. Historically, fermented dairy products have been used as convenient vehicle for the consumption probiotic. However, addressing the potential drawbacks of fermentation has recently led to increase in research on probiotic dairy drinks prepared without fermentation. These non-fermented dairy drinks have the advantage of maintaining the original flavors of milk drinks, containing potential health functional probiotics, and being an alternative dairy product that is helpful for probiotics intake. Currently, research on plant-based dairy products is rapidly increasing in the market. These developments might suggest the potential for novel forms of non-fermented dairy beverages with substantial prospects in the food market. This review aims to provide an overview of milk-based dairy beverages, both fermented and non-fermented, and discuss the potential of non-fermented dairy products. This exploration paves the way for innovative approaches to deliver probiotics and nutrition to consumers.

Key words: probiotics, dairy product, milk drink, non-fermented milk drink

Introduction

Owing to the rapidly changing environment, consumer have recently become interested in consumption of functional food to improve and maintain their health (Kuster-Boluda and Vidal-Capilla, 2017; Lillo-perez et al., 2021). Functional foods are considered to promote health and wellbeing and reduce diseases. To provide these benefits, functional foods should also contain health-promoting components such as probiotic, prebiotics, vitamins, minerals, bioactive compounds, and antioxidants (Konstantinidi and Koutelidakis, 2019; Mantzourani et al., 2019). Probiotics and probiotic-based foods are consumed worldwide as functional health foods (Lee and Paik. 2021; Lillo-perez et al., 2021).

Probiotics defined as live microorganisms that confer health benefits to the host when administered at adequate dose (FAO and WHO, 2002). Probiotics have been shown to exhibit antioxidant, antidiabetic, anticancer, anti-inflammatory, anticavity, neuroprotective, immune-enhancing, antihypertensive, and cholesterol-reducing properties (Cheon et al., 2020; Jang et al., 2018; Kim et al., 2021; Lewis-Mikhael et al., 2020; Lim et al., 2020; Song et al., 2019). Recently, one study reported that probiotics as prophylactics or adjuvant treatment, could help heal COVID-19 (Lee and Paik, 2021), and many people have become more concerned about their health since the outbreak of COVID-19. The interest in fermented foods has increased significantly since COVID-19, while fermented foods alone may not offer complete protection against viral diseases such as COVID-19. However, it is reported that they could have a potential role in supporting the immune system and aiding in its defense (Wan-Mohtar et al., 2022).

Fermentation is an age-old food processing technique known to enhance the value of food by breaking down complex organic compounds through biochemical transformations (Romulo and Surya, 2021). This technology has been utilized extensively in dairy product

processing with various health benefits (Ilango and Antony, 2021). In addition, most probiotic vehicles are probiotic foods by fermented or non-fermented to improve for the host (Rodrigues et al., 2019). Several food products such as dairy, beverages, meats, and cereals have been used as delivery vehicles for probiotics (Aspri et al., 2020). Among these products, probiotic dairy products are the most common because of their convenience of consumption, nutritional, and physicochemical properties, intestinal regulation, and therapeutic effects (Yilmaz-Ersan et al., 2020). Nevertheless, research is being conducted to solve problem caused by the use of probiotics in dairy products due to various side effects by probiotics and taste aversion due to fermentation (Sotoudegan et al., 2019).

In general, fermented dairy products are well known for their health benefits; however, non-fermented dairy products have also been reported. Future trends in the food industry will continue to develop with the increasing interest in functional foods. In particular, new probiotic-containing dairy products can developed with various functionalities.

This review aims to provide the currents and prospects for fermented and non-fermented milk drinks. It includes an overview of probiotics, characteristics of fermentation and non-fermentation, and detailed discussion about milk-based drinks, which encompass both fermented and non-fermented varieties. Furthermore, we comprehensively explore the potential for non-fermented dairy products to become more widely used as functional foods in the future, owing to their health benefits and convenience.

Probiotics and Its Applications

The concept of probiotics was introduced in 1908 by the Russian Nobel laureate Elie Metchnikoff (Zendeboodi et al., 2020). Metchnikoff observed that consuming fermented foods, particularly those containing lactic acid bacteria (LAB), had favorable effects on

human health and contributed to longevity (Cremon et al., 2018). As the guideline by Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microbial cultures that confer health benefits to the host when administered in adequate amounts (FAO and WHO, 2002; Garcia-Burgos et al., 2020; Rasika et al., 2021). It is important that probiotics is considered safety issues when selecting probiotics strains (Shi et al., 2016). For instance, the identification of virulence factors related to pathogenicity and infectivity, virulence and metabolic activity of microorganisms (Shi et al., 2016). Probiotics promote the gastrointestinal microbes by competing with harmful bacteria (El-Saadony et al., 2021;Tripathi and Giri, 2014). In general, LAB are considered to be representative probiotics, and many studies have reported their roles in the prevention of antibiotics-related diarrhea, irritable bowel syndrome, and immune modulation (Kim et al., 2021). In addition, probiotics has been reported to have various bio-functional benefits such as antioxidant, anti-inflammatory, anticancer, anticavity, and antimicrobial effects, as well as improving intestinal function (Hyun et al., 2023; Kang et al., 2023; Kim et al., 2021; Wang et al., 2022).

Dairy based and non-dairy based products that contain probiotics can provide these benefits. Most importantly, dairy-based products are widely consumed, consumer interest in health has increased worldwide increase in recent years, and global market trends shows challenges following consumer needs (Garcia-Burgos et al., 2020; Ozuna and Franco-Robles, 2022; Yilmaz-Ersan et al., 2020). The health functionality of fermented dairy products, as well as non-fermented dairy products, has shown potential for development (Jang et al., 2022; Oliveira et al., 2017). In addition, although dairy product with sugar and additives are the most common products for consuming probiotics, one study showed that the development of probiotic milk without sugar and food additives might address in intent of the government and consumers to reduce the consumption of highly processing foods (Oliveira et al., 2017).

In addition to dairy-based drinks, probiotic drinks are made from diverse raw materials, including cereals, fruits, and vegetables (Chavan et al., 2018). In recent, the development of non-fermented probiotic beverages as new functional product has also been reported without fermentation-induced changes (De Oliveira Ribeiro et al., 2020). Many consumers prefer not to consume dairy products such as milk, yogurt, cheeses, and ice cream because of lactose intolerance, allergies, restricted diets, and vegan diets (De Oliveira Ribeiro et al., 2020; Szparaga et al., 2019). Therefore, non-fermented beverages have been studied using plant-based milk, fruit, and other ingredients (Szparaga et al., 2019;). Importantly, the food matrix should be carefully chosen to maintain probiotic viability, retain probiotic activity, and reach the intestine at sufficient levels (FAO and WHO, 2002). One study showed that fruits being used as vehicles for probiotics because they contain diverse minerals, vitamins, antioxidants, and dietary fibers (Santos et al., 2017). Non-dairy milks, such as soy bean, almond, and coconut milk are good alternative substances for the development of probiotic beverage owing to their suitable food matrices (Santos et al., 2017).

Future trends in the food industry will continue to develop with the increasing interest in functional foods. In particular, new probiotic-containing dairy products can be developed with various functionalities.

Fermentation and Non-fermentation

Fermentation is one of the oldest technologies used to preserve and create value-added advantages. Therefore, fermentation is reported to be an economical technology (Agyei et al., 2020; Garcia-Burgos et al., 2020; Ranadheera et al., 2017; Shori, 2016). There are two primary methods for fermenting foods. One method involves natural fermentation, known as “wild fermentation” or “spontaneous fermentation,” which takes place with microorganisms

naturally present in the raw food or processing environment. The other technique is the addition of starter cultures, also termed “culture-dependent ferments,” to foods (Patel et al., 2023). Generally, fermentation enhances the nutritional value and sensory perceptions through microbial metabolic activities, thereby increasing the overall value of the food (Shori, 2016).

Fermentation is a biological process in which microorganisms break down complex organic compounds into simpler forms. Enzymatic or microbial actions on food constituents drive the fermentation process, inducing favorable biochemical alterations that substantially transform the food (Nkhata et al., 2018; Sikic-Pogacar et al., 2022). Fermentation is a natural means of enhancing the content of vitamins, essential amino acids, proteins; mitigating anti-nutrients; elevating the visual appeal of food; enriching flavors; and intensifying aromas (Sikic-Pogacar et al., 2022). Consequently, microbial activity plays a pivotal role in shaping the fermentation of edibles and precipitating shifts in their chemical and physical attributes. Various benefits of fermented foods made through fermentation have been reported (Sanlier, 2019).

Traditionally, probiotics have been widely used to ferment dairy products as starter cultures, and most commercial probiotics include *Lactobacillus* sp., *Bifidobacterium* sp., and *Streptococcus thermophilus* (Garcia-Burgos et al., 2020; Ranadheera et al., 2017; Senok et al., 2005; Shori, 2016). Dairy products align with probiotics because they are traditionally associated with beneficial fermented bacteria and fermented dairy products. Consumers naturally relate fermented dairy products with living cultures and recognize the benefits of these cultures (Nagpal et al., 2011). Various health benefits of dairy product and fermented foods have been reported, including modulation of the intestinal microbiota and immune-enhancing, antibacterial, anticancer, anti-allergenic, and antioxidant effects (Abd Rahim et al., 2023; Garbacz, 2022; Jang et al., 2018; Kariyawasam et al., 2021; Macro et al., 2017;

Tasdemir and Sanlier, 2020). However, the health benefits of dairy products vary depending on the microbial species (Widyastuti et al., 2021). For example, *Lactobacillus helveticus* potentially impacts human well-being through direct mechanisms, such as inhibiting pathogens, altering gut microbiota, and influencing the host immune system (Widyastuti et al., 2021).

Many studies have shown that probiotics grow stably in food and are known to have various advantages, such as physicochemical, stability-improving, nutritional, sensory, and functional properties (Ranadheera et al., 2017) . Nevertheless, consumers strongly demand non-fermented products; therefore, the development and sales of non-fermented drinks are expected to increase (Ranadheera et al., 2017). Commonly, in fermented products, the viability of numerous probiotic strains could be compromised due to the antagonistic interplay between starter cultures and probiotics, alongside the acidification inherent to these cultured products. Consequently, a novel trend has surfaced: non-fermented dairy products have been developed and brought to the market, reflecting this evolving approach (Awaisheh, 2012).

To date, limited studies on non-fermented drinks have been reported. The characteristics of the advantages and disadvantages of non-fermented milk drink are shown in Table 2. The reasons for their benefit can be summarized as follows. 1) Incorporating probiotics into non-fermented dairy products allows individuals to access the potential health benefits associated with probiotics while retaining the original flavor and texture of the dairy products. 2) The addition of probiotic to non-fermented dairy products can target individuals who might prefer milder flavors or who may not be accustomed to the tangy notes often associated with traditional fermented dairy products. By introducing probiotics into non-fermented products, individuals can experience the advantages of probiotic consumption without altering their taste preferences. Furthermore, some consumers might have dietary or cultural considerations

that lead them to opt for non-fermented dairy products. These individuals benefit from the nutritional potential of probiotics without compromising on their dietary choices or culture culinary practices. Ultimately, the inclination towards using probiotics in non-fermented dairy products underscores the evolving landscape of health-conscious consumer preferences. The reflects the desire to integrate wellness-promoting elements into a diverse range of foods catering to a spectrum of tastes, preferences, and dietary requirements.

Dairy-based Milk Drink

Dairy-based milk drinks were divided into fermented and non-fermented products. Each has its own characteristics and related probiotics strains health properties have been reported (Table 1).

Fermented milk drink

Fermented milk is an important part of the human diet worldwide because of its high nutritional value and enhancement of sensory factors through fermentation (Shori, 2016). Fermented milk is the most commonly used probiotic products delivering probiotics (Ranadheera et al., 2017; Shori, 2016). The most common examples of fermented milk include yogurt, kefir, acidophilus milk, and any other products; however, there are many different products based on geography, historical practices, and diverse types of milk (Bagheripoor-Fallah et al., 2013; Savaiano and Hutkins, 2020).

Although several viable organisms are required in probiotic products, either to ferment or contain them, the most commonly used strains are *Lactobacillus* sp., *Lacticaseibacillus* sp., *Limosilactobacillus* sp., *Lactiplantibacillus* sp., *Ligilactobacillus* sp., *Lactococcus* sp., and *Bifidobacterium* sp. The most commonly used *L. acidophilus*, *L. gasseri*, *L. helveticus*, *L.*

delbrueckii subsp. *bulgaricus, L. casei, L. paracasei, L. rhamosus, L. fermentum, L. reuteri, L. plantarum, Lc. lactis*; *Bifidobacterium* sp. include *B. bifidum*, *B. longum*, *B. breve*, and *B. animalis* ssp. *lactis* (Kandylis et al., 2016; Yerlikaya, 2014). However, milk is fermented by each special culture (Garcia-Burgos et al., 2020). *Lactiplantibacillus plantarum* and *Bifidobacterium* sp. are frequently selected as probiotics and are well suited for the production of fermented milk products. Fermented milk by *L. plantarum* showed high antioxidant effects and 89% angiotensin-converting enzyme (ACE) inhibitory effects (Chen et al., 2018; Li et al., 2020).

Yogurt is an excellent source of nutrients, highly nutritive proteins, and bioactive peptides formed by fermentation. Fermentation of lactose by yogurt culture enhances digestibility for patients with lactose intolerance in comparison to milk (Meybodi et al., 2020). Generally, *Lactobacillus* sp. and *Streptococcus thermophilus* are used as starter cultures, and yogurt is manufactured containing various probiotic strains (Jang et al., 2018). Additionally, yogurt has been reported to various health benefits such as gastrointestinal effects, increased digestibility of lactose, heart health improvements, anti-cholesterol effects, anti-cancer effect, immune-enhancing effects, and reduction of type 2 diabetes (Daniel et al., 2022; Freitas, 2017; Gharibzahedi and Chronakis, 2018; Hazra et al., 2013).

Kefir is a naturally fermented milk product produced using kefir grains, or mother cultures prepared from kefir grains. Kefir grains are a good source of LAB, acetic acid bacteria, and various yeasts cells combined with a matrix of casein and complex sugars in a polysaccharide matrix (Ahmed et al., 2013). Kefir has reported to have numerous positive effects, such as wound healing, anti-carcinogenic, immunomodulatory, and antimicrobial effects, and has also been reported to inhibit *Helicobacter pylori* (Arslan, 2015; Bourrie et al., 2016).

Kumys is a fermented milk drink that is popular in Central Asia and Eastern Europe (Kim et al., 2017). Kumys is naturally fermented by LAB and yeast (Li et al., 2022). *Lactobacillus* strains derived from kumys have shown potential as probiotics (Wu et al., 2009). *L. fermentum* SM-7 in kumys reduced cholesterol levels in both *in vitro* and *in vivo* experiments (Pan et al., 2011). In addition, kumys has been suggested to be a therapeutic agent for asthma, cardiovascular disorder, and gynecological diseases (Yerlikaya, 2014).

Acidophilus milk is a type of fermented milk product that uses *L. acidophilus* as the starter culture. Acidophilus milk can be fermented or non-fermented (Aryana and Olson, 2017). One study reported that fermented milk with *L. acidophilus* LA-5 reduced the number of pathogenic bacteria and the beneficial bacteria protected against intestinal diseases (Meng et al., 2021).

Non-fermented milk drink

Usually, milk is a well-known nutritious food containing bioactive compounds, such as immunoglobulin, antimicrobial peptides, enzymes, cytokine, and other substances and essential nutrients. It has played an important role in promoting health and well-being (Gorska-Warsewicz et al., 2019; Jung et al., 2016; Khan et al., 2019). In addition, milk has been reported to have health benefits, such as bone health, enhanced immune system, improved intestinal health, reduced risk of stroke, cancer, and high blood pressure, and improved weight management (Chauhan et al., 2021; Jung et al., 2016).

In the food industry, various functional foods have been developed by applying probiotics to milk as food additives (Damian et al., 2022). However, in recent decades, the market for probiotic dairy products has mainly consisted of fermented products such as yogurt, fermented milk, and cheeses (De Oliveira Ribeiro et al., 2020). Generally, probiotic milk is a new product of fermentation that is based on a widely used food technology and enhances the

availability of nutrients and bioactive compounds (Garcia-Burgos et al., 2020; Vicenssuto and de Castro, 2020). Recently, the government and consumers interested in the development of probiotic milk have shown great interest with the aim to reduce the consumption of highly processed products (Oliveira et al., 2017). And as another type of probiotic milk for health-conscious people, non-fermented probiotic milk has become excitingly popular (Jang et al., 2022). The process of non-fermented milk drink is shown in Figure 1.

Non-fermented milk refers to milk containing probiotics without fermentation process. Therefore, non-fermented milk drink has several advantages compared to fermented milk drink. Firstly, the original flavor of milk can be maintained, secondly, since probiotics containing potential functionality are added, the product itself can be expected to be functional, and lastly, it can be expected to be another alternative food for consuming probiotics (Table 2). Additionally, Table 2 shows the advantages as well as disadvantages of non-fermented milk drink.

Several research articles have been reported non-fermented milk and its potential effects, follow as: milk containing *L. acidophilus* maintains its physicochemical, microbiological, and sensory characteristics. Additionally, a study reported strain screening to develop non-fermented milk containing probiotics and the physicochemical, microbiological, sensory, and functional properties of non-fermented milk (Aboulfazli et al., 2016; Jang et al., 2022; Oliveira et al., 2017).

Jang et al. (2022) reported that non-fermented milk with probiotics showed antioxidant effects depending on the health functionality of the added probiotics compared with milk without probiotics. Nevertheless, these results will provide sufficient supplementary data for future research on non-fermented milk with probiotics. In addition, non-fermented milk might be a new dairy product that could conveniently deliver probiotics to the host. Furthermore, the development of various non-fermented milk products with added sugar or

food additives is expected to complement the benefits of fermented milk (Jang et al., 2022; Oliveira et al., 2017). In addition, the use of ultrasound could lead to the production of functional non-fermented milk with health benefits by reducing the particle size, enzymatic activities, and modification of proteins (Kaveh et al., 2023).

Conclusion and Prospect

The probiotic product industry is growing globally owing to various reported health functionalities. In particular, the dairy industry has been reported to be a top trend in the food industry. Generally, dairy products undergo a fermentation process to protect against contaminants and maintain viability and functionality during their shelf life. In addition to traditional probiotics, the integration of new functional ingredients such as prebiotics, postbiotics, and synbiotics is gradually gaining attention. This combination may further enhance the health-promoting potential of fermented dairy products. Fermentation process is one of the oldest technologies used to improve sensory properties and nutrition benefits. However, in addition to dairy-based fermented products, plant-based fermented products, such as soy- and cereal-based products, will be developed. Products that develop similar flavors or emphasize the natural flavor of plants will continue to be perceived as part of animal dairy products.

Although dairy-based fermented products are considered the most common products that deliver probiotics to the host for health purposes, dairy-based non-fermented products could be developed and commercialized in the coming future. Previous studies have reported the physicochemical, microbiological, sensory, and health benefits of non-fermented milk. For non-fermented dairy products to be commercialized, probiotic viability and safety during storage period, intestinal survival rates, and various health functional studies must be further

conducted. Non-fermented dairy products can be fortified with probiotics to provide health benefits without altering their original characteristics. Similar to fermented dairy products, the rise in plant-based milk alternatives such as oat, pea, and rice milk, is driving the creation of non-fermented dairy products. Non-fermented products are enriched with probiotics and functional ingredients, such as vitamins, minerals, omega-3 fatty acids, and antioxidants, to offer enhanced nutritional value. Lastly, with growing health consciousness, non-fermented dairy products are adapting to consumer preferences by offering reduced sugar and low-fat additives while maintaining flavor and texture.

Taken together, fermented and non-fermented dairy product could be used as important foods that provide probiotics to humans. Research on the functionality and benefits of non-fermented dairy products is limited; however, the benefits and functional aspects of these products render them a potentially successful new type of product in the dairy industry and provide exciting developments that cater to diverse tastes, dietary needs, and wellness aspirations.

Conflict of Interests

All authors declare no conflict of interest.

Author Contributions

Conceptualization: Jang HJ, Lee NK, Paik HD. Investigation: Jang HJ, Lee NK. Writing – original draft: Jang HJ. Writing – review & editing: Jang HJ, Lee NK, Paik HD.

Ethics Approval

The article dose not require IRB/IACUC approval because there are no human and animal participants.

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629 **Table 1. Fermented and non-fermented dairy-based probiotic milk drinks and their health properties**

Process type	Probiotic strain	Health properties	Reference
Fermented			
Acidophilus milk	*L. acidophilus*	Improvement of gastrointestinal conditions	Hati and Prajapati, 2022
Bifidus milk	*B. longum*; *B. bifidum*	Treatment of gastrointestinal conditions and constipation	Yerlikaya, 2014
Fermented milk	*L. acidophilus*; *L. rhamnosus*; *L. fermentum*; *L. plantarum*; *L. paracasei*; *L. casei*; *L. delbruekii*; *L. brevis*; *S. thermophilus*; *B. bifidum*	Antioxidant effect; antimicrobial effect; antihypertensive effect; reduction of LDL; anticholestrol effect; anticarcinogenic effect; and antiobesity effects	Beltran-Barrientos et al., 2016; Hou et al., 2019; Mendez et al., 2019; Oliveira et al., 2001; Wa et al., 2019
Kefir	*L. casei*; *L. aciophilus*; *L. paracasei*; *L. fermentum*; *K. marxianus*; *S. unisporus*; *Sacch. cerevisiae*	Antimutagenic effect; anticarcinogenic effect; cholesterol-lowering; reducing lactose intolerance; immune system modulation; antioxidant effect; and antimicrobial effects	Bengoa et al., 2019; Egea et al., 2022; Hong et al., 2019; Melo et al., 2018; Nielsen et al., 2014; Otles et al., 2003
Kumys	*L. delbrueckii*; *K. marxianus*	Anti-asthma effect, supports the cardiovascular system, and inhibition of *Helicobacter pylori*	Arslan, 2015; Garcia-Burgos et al., 2020; Marsh et al., 2014; Yerlikaya, 2014
Yogurt	*L. acidophilus*; *L. bulgaricus*; *L. rhamnosus*; *L. plantarum*; *L. helveticus*;	Improvement of gastrointestinal; antimicrobial effect;	Arain et al., 2023;

	L. casei; *L. fermentum*; *S. thermophilus*; *B. lactis*	lowering the cholesterol; reducing lactose intolerance; anticancer effect; immune system modulation; improvement of inflammatory bowel disease effect; and antidiarrheal effects	Garcia-Burgos et al., 2020; Ghasempour et al., 2020; Lim et al., 2020; Olson and Aryana, 2022; Shah, 2006; Tasdemir and Sanlier, 2020
Non-fermented			
Milk drink	*L. plantarum*; *L. acidophilus*; *B. lactis*; *B. animalis*	Improvement of gastrointestinal conditions and antioxidant effects	Daneshi et al., 2013; Jang et al., 2022; Oliveira et al., 2017

630 *L., Lactobacillus*; *Lacticaseibacillus; Lactiplantibacillus; S., Streptococcus*; *B., Bifidobacterium*; *K., Kluyveromyces*; *Sacch. Saccharomyces*.
631

632

633 **Table 2. Characteristics of the advantages and disadvantages of non-fermented milk**

634 **drink compared to fermented milk**

Advantages	Disadvantages
• Retaining the original flavors of milk drink • Containing the potential health benefits of probiotics • Alternative dairy products to help you consume probiotics	• Problems with storage stability due to microbial growth • Difficulty obtaining nutritional benefits due to material decomposition, which is an advantage during fermentation

635

Figure legends

Fig. 1. Manufacturing processes of fermented and non-fermented dairy products.

Fig. 1.