Chapter 6

Viability of Probiotics in Dairy Products: A Review Focusing on Yogurt, Ice Cream, and Cheese

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Abstract

Probiotic is a dietary supplement of live microorganism that contributes to the health of the host. Commercially produced food biotechnology products may contain either a single probiotic strain or bacterial mixtures of various complexities to increase food nutritional and therapeutic properties. It is highly desirable that the viable number of probiotics in the final product to be at least 10^6–10^7 cfu/g to be accepted as the therapeutic minimum. Various ways were carried out to enhance the viability of probiotics. Therefore, the purpose of the present study is to review the importance of probiotics in dairy food and their viability in yogurt, ice cream and cheese during storage.

1. Introduction

Foods are functional when they provide additional properties other than nutritive values. Dairy products are established as healthy natural products and they form one of the four major food groups that make up a balanced diet [1]. Regular consumption of certain dairy products has beneficial effects in the prevention of disease [2] because they contain a number of active compounds with putative roles in both nutrition and health protection such as minerals, fatty acids, prebiotics, probiotics, carbohydrates and proteins/peptides.

Lactic Acid Bacteria (LAB) are friendly bacteria associated with the human gastrointestinal tract. Most of them are important as probiotic microorganisms. They are strictly fermentative dependent on carbohydrates for their energy supply and produce lactic acid from the carbohydrate catabolisms which is the major end-product of sugar fermentation. These bacteria are gram-positive, rod-shaped, non-spore-forming, catalase-negative organisms that are devoid of cytochromes and are of non-aerobic habit but are aero-tolerant, fastidious, acid-tolerant.

LAB and their metabolites play a key role in enhancing microbiological quality and shelf life of fermented dairy products [3,4]. LAB has an essential role in most fermented food for their ability to produce various antimicrobial compounds promoting probiotic properties [5].

The probiotic is living microbial feed supplements added to the diet [6]. It is now popularly referred to as being a mono-or mixed culture of live microorganisms e.g. as dried cells or as a fermented product. Common probiotics in use include *Bifidobacterium* spp. and members of LAB such as *Lactobacillus* species **(Table 1)**. These bacteria are added to fermented milk because they help to improve the balance of the intestinal microflora of the host upon ingestion [7,8]. In addition these probiotics contribute to the development of the immune system, improvement of normal intestinal morphology and maintaining a chronic and immunological balanced inflammatory response [9]. The growth of these probiotics showed inhibitory activities toward the growth of pathogenic bacteria via the creation of inhibitory compounds such as bacteriocins or reuterin, hydrogen peroxide, reduced pH as a result of accumulation of organic acids and competitive adhesion to the epithelium [10]. Probiotics also produce enzymes that help in the digestion of food in addition to B-complex vitamins production and neutralization of pathogenic microorganisms responsible for infections and diarrhea [11,12].

Viability and metabolic activity of the bacteria are important considerations in probiotic inclusion in foods. This is because the bacteria need to survive in the food during shelf life and gastrointestinal digestion i.e. acidic conditions of the stomach and degradation by hydrolytic enzymes and bile salts in the small intestine [13]. To ensure health benefits can be delivered by food containing probiotics, products sold with any health claims must meet the standard of a minimum level for probiotic bacteria ranging from 10^6 to 10^7 cfu/ml at the expiry date [14]. Therefore, the purpose of the present study is to review the importance of probiotics in dairy food such as yogurt, ice cream and cheese and their viability in these products during storage.

2. Probiotics

The word probiotic, derived from the Greek language, means for life is defined as ‘living microbial feed supplements added to the diet and offer beneficial effects on the host by enhancing their intestinal microflora balance’ [6]. It is now popularly referred to as being a mono- or mixed culture of live microorganisms (e.g. as dried cells or as a fermented product) which usefully effects the host by enhancing the properties of the native microflora [15]. Common probiotics in use include *Bifidobacterium* spp. members of LAB and selected species of yeasts. To complement probiotics, “prebiotics” defined as selective non-digestible carbohydrate food sources, are becoming increasingly used in promoting the proliferation of bifidobacteria and lactobacilli [16].

3. Therapeutic Value of Probiotic in Dairy Food

3.1. Control of intestinal infections

Probiotic bacteria such as lactobacilli and bifidobacteria have antimicrobial activity [17]. Both *L. acidophilus* and *B. bifidum* for instance inhibit numerous of the generally known food borne pathogens [18-20]. The consumption of milk cultured with *L. acidophilus* or *B. bifidum* or both for preventative control of intestinal infections [19] can be occurred via:

- Inhibitory/antimicrobial substances production such as hydrogen peroxide, bacteriocins, organic acids, antibiotics and deconjugated bile acids.
- Competitive antagonist’s action for example, through competition for adhesion sites and nutrients.
- Immune system stimulation.

The organic acids produced by the probiotics caused reduction in the pH and change the oxidation reduction potential in the intestine which leading to antimicrobial action. In addition, the limited oxygen content in the intestine can help the organic acids to inhibit especially pathogenic gram-negative bacteria type’s e.g. coliform bacteria [21-23].

3.2. Reducing lactose intolerance

The lack of *β-D*-galactosidase in the human intestine results in the inability to digest lactose adequately follows by different degrees of abdominal pain and discomfort [24]. LAB used as starter cultures in milk during fermentation and probiotic bacteria such as *L. acidophilus* and *B. bifidum* produce *β-D* –galactosidase that digest lactose which helps consumers having better tolerance for fermented-milk products [24]. This utilization is referred to intra-intestinal digestion by *β-D*-galactosidase. Increased digestion of lactose may not only occur by hydrolysis of the lactose before consumption, but also in the digestive tract after ingesting of milk containing *L. acidophilus* [24]. Thus the continued utilization of lactose inside the gastrointestinal tract is governed by the survival of the lactobacilli.

3.3. Reduction in serum cholesterol levels

The consumption of fermented milk could significantly reduce serum cholesterol [25]. This is good news for hypercholesterolemic persons since substantial decrease in plasma cholesterol level plays a role in reduction heart attacks risk [26]. Appreciable amounts of cholesterol metabolism occur in the intestines before passage to the liver. This could provide some explanation on the association between the presence of certain *L. acidophilus* strains and some *bifidobacteria* species with the ability to reduce cholesterol levels inside the intestine. Cholesterol co-precipitates with de-conjugated bile salts as the pH drops as a result of lactic acid production by LAB [27]. The role of *bifidobacteria* cultures in reducing serum cholesterol is poorly known. Feeding of *bifidobacteria* to rats reduced serum cholesterol which may involve HMG-CoA reductase [28]. Sudha et al. [29] suggested a factor is formed in the milk during fermentation that inhibits cholesterol synthesis in the body. Alternatively, *L. acidophilus* may de-conjugate bile acids into free acids which are excreted faster from the intestinal tract than are conjugated bile acids. Subsequently, the production of fresh bile acids from cholesterol can decrease the total cholesterol level in the body [27]. A third hypothesis is that at lower pH values the production of lactic acid by LAB resulted in co-precipitation of cholesterol with de-conjugated bile salts cause reduction of cholesterol [29].

3.4. Anti-carcinogenic activity

probiotics are known to have antitumour action related to the inhibition of carcinogens and/or inhibition of bacteria that convert pro-carcinogens to carcinogens [19,30], improvement of the host's immune system [22,31] and/or reduction of the intestinal pH to decrease microbial activity. Studies in rats showed that probiotic bacteria in yogurt and fermented milk inhibited tumor formation and proliferation [19,30].

3.5. Prevention of colon cancer

Probiotics have shown capability to reduce risk of colon cancer owing to their ability to bind with heterocylic amines; carcinogenic substances that formed in cooked meat [30]. Most human studies have reported that probiotic may apply anti-carcinogenic effects by reducing the activity of ß-glucuronidase, an enzyme which produces carcinogens in the digestive system [32]. Although human intervention studies demonstrate the reduced presence of biomarkers associated with colon cancer risk. The evidence that probiotics decrease colon cancer occurrence in humans is lacking [33]. Thus the subject of probiotic uptake and cancer prevention is still open to further investigation.

3.6. Anti-diarrhea effects

Diarrhea can have many causes and its effects on flushing out the bacteria living in the intestine leaves the body vulnerable to opportunistic harmful bacteria. It is important to replenish the body with probiotics during and after the incidence of diarrhea. The advantages of probiotics in the inhibition and treatment of a range of diarrhea illnesses, such as acute diarrhea caused by rotavirus infections, antibiotic-associated diarrhea, and travelers' diarrhea have been extensively studied [34]. LAB may possibly reduce diarrhea in some ways including competition with pathogens for nutrients and space in the intestines [34]. For instance *L. casei* and *B. bifidum* effectively prevent or treat infantile diarrhea [34] by several ways:

1) Compete with pathogens for nutrients and space in the intestines.
2) Some metabolism by-products such as acidophilin and bulgarican produced by *L. casei, L. acidophilus* and *L. bulgaricus* have a direct effect against inhibition of pathogens growth.
3) Enhance immune system which has effect against diarrhea, particularly through alleviation of intestinal inflammatory responses and intestinal immunoglobulin A (IgA) responses which cause create gut-stabilizing effect [31,34].

3.7. Improving immune function and preventing infections

Lactic acid bacteria are assumed to have some valuable effects to enhance immune function. These include the improvement of immune function by increasing the number of IgA producing plasma cell, increasing or educating phagocytosis other than increasing the proportion of T lymphocytes and natural killer cell [34]. They may protect against pathogen and to prevent or treat infections such as postoperative infections [35], respiratory infections [36], and the growth of *Helicobacter pylori,* a bacterial pathogen responsible for type B gastritis and peptic ulcers.

3.8. Anti-inflammatory effects

Probiotics have been shown to modulate inflammatory and hypersensitivity reactions. They can affect the intestinal flora and may have beneficial effects in inflammatory bowel disease (IBD), which includes ulcerative colitis, Crohn's disease and pouchitis [34]. Clinical studies suggest that they can prevent reoccurrences of IBD in adults [34], enhance remediation of milk allergies and decrease the risk of atopic eczema in children [37].

4. Application of Probiotics in Dairy Foods

Growing consumer knowledge of roles of diet in health has aroused amongst others the demand for foods containing probiotic. A number of dairy food products including frozen fermented dairy desserts [38], yogurt [39], cheeses [40], freeze-dried yogurt [41], ice cream [42] and spray dried milk powder [40] have been utilized as delivery vehicles for probiotic to consumer. Hence the selection and balancing of LAB is important to ensure food and dairy products maintain their desirable flavor, texture and nutritional value characteristics, because these parameters may be affected by the initial composition of the milk flora and starter culture [43].

To elicit health effects, probiotic organisms must be viable (~ 10^9 cfu/ day) at the time of consumption [44]. Therefore, it is important to minimize the decline in the numbers of viable bacteria during storage period. Dairy foods present ideal delivery system of food for probiotics to the human gut because it offers suitable environment and nutrients to promote growth or support viability of these cultures. The fermented dairy products are the most popular food delivery systems for probiotic. However the low pH, the presence of H_2O_2 and inhibitory substances produced by the bacteria and the aerobic conditions of production and packaging may result in the decreases in the survival of probiotics in the final product. In fact the required level of viable cells of probiotic bacteria in many commercial dairy products cannot be guaranteed and therefore, failed the prerequisite for successfully delivery of probiotics [45].

5. Yogurt

The most common functional dairy products are those containing probiotic bacteria, quite frequently enriched with prebiotics, such as yogurt [46]. Yogurt is fermented milk obtained by lactic acid bacteria fermentation of milk and is a popular product throughout the world. It is recognized as a healthy food due to the beneficial action of its protein and its rich contents of potassium, calcium, protein and B vitamins.

Yogurt is formed during the slow fermentation of milk lactose by the thermophilic lactic acid bacteria *S. thermophilus* and *L. delbrueckii ssp. Bulgaricus*. However, these bacteria are not indigenous to humans and cannot colonize the intestine to promote human health. Thus probiotics, mainly *Lactobacillus acidophilus* and *Bifidobacterium* spp. are added to improve the fermentation process for production probiotic yogurt [47] and offer many advantages for the consumer. *S. thermophilus* and *L. delbrueckii ssp. Bulgaricus* are required to convert milk to yogurt whereas *L. acidophilus* and *Bifidobacterium* are added to increase the functional and health-promoting properties. Some researchers proposed that yogurt containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* could be regarded as members of the probiotic because both bacteria provide health benefits to the host [48]. These bacteria are able to release β- galactosidase enzymes that improve the digestion of nutrients in the intestine and modulate immune responses for human health [49].

The food biotechnology industry has in recent years developed a huge number of commercial products containing a single probiotic strain or bacterial associations of various complexities [18,50]. The development of yogurt with new flavors and products with health benefits has the potential to increase sales and to consumers satisfaction. Yogurts in the marketplace are available to satisfied different consumer groups. For example, fat free dairy products for consumers with cardiovascular problems and lactose free dairy products for lactose intolerant people. In addition, folic acid enriched yogurt taken during initial stages of pregnancy help to prevent neural tube defects such as anencephaly, spina bifida, heart defects, facial clefts, limb deficiencies and urinary tract abnormalities [51].

6. Viability of Probiotic in Yogurt

Commercially produced food biotechnology products may contain either a single probiotic strain or bacterial mixtures of various complexities. Thus, the addition of probiotic increases yogurt's nutritional and therapeutic properties [52]. It is important that probiotic yogurt must contain living probiotic strains in adequate concentration at the time of consumption [14]. However, the key problems associated with incorporating probiotic bacteria into milk during fermentation are slow growth in milk and low survival rate during storage [53]. One of the strategies applied to improve the growth of probiotic bacteria is the addition of prebiotic substances with proper selection of starter cultures [53,54]. In order to provide functional properties and additional nutrients for bacteria growth in probiotic yogurts many other supplements with active components have been studied such as plant extracts, phenolic compounds and antioxidative substances [55-59]. Recently, cocoa powder and stabilizers are used as natural food additives to increase the survival of probiotic bacteria during passage through gastric tract [60]. In addition, lipid fraction of cocoa butter found to protect *B. longum* from environment stress [61]. Chocolate can also enhance the survival of *L. helveticus* and *B. longum* (91% and 80% respectively) compared to milk (20% and 30%) in low pH environment [62].

Several studies have demonstrated the effect of phenolic compounds on the growth and metabolism of probiotic in yogurt [60-62]. The bacterial species and strain in addition to chemical structure and concentration of the polyphenols play a significant role in sensitivity of probiotic to the phenolic compounds [63]. *L. plantarum* and *L. casei* Shirota strain found to be able to metabolize phenolic compounds [64,65]. Kailasapathy et al., [66] reported that the amount 5 or 10 g/100 g of added fruit mixes (mango, mixed berry, passion fruit and strawberry) in yogurt did not affect *B. animalis* ssp. *lactis* LAFTIs B94 growth except on *L. acidophilus* LAFTI L10 yogurt with 10 g/100 g passion fruit or mixed berry. However, the reduction in *L. acidophilus* counts was higher than the plain yogurt ($p<0.01$) which could be related to the chemical composition of these fruits. On the other hand, [67] found that the addition of passion fruit peel powder (0.7 g/100g) had no significant effect on viability of *L. acidophilus* LAFTI L10 in yogurt during 28 days of storage. The differences between the amounts of added passion fruit in previous studies could explain the discrepancy in the probiotic viability results obtained. Previous study observed that immobilized *L. casei* cells on fruit pieces (apple or quince) could be promising application in dairy food processing [68]. Immobilized *L. casei* cells on fruit pieces found to supports further the chances of *L. casei* survival for a long period of storage up to 129 days and can be adapted to the acidic condition which usually acts as inhibitor towards bacteria growth [68].

Chromatographic studies were used to evaluate the effect of *S. thermosphilus* and *L. bulgaricus* in yogurt on six phenolic compounds Catechin gallate (CG), epigallocatechin (EGC), catechin (C), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG) and epicatechin gallate (ECG) in green and black teas [69]. The chromatographic profiles of green and black tea phenolic compounds after the treatment with *S. thermosphilus* and *L. bulgaricus* yogurt bacteria showed no significant alteration ($p<0.05$) of these phenolic compounds compared to before treatment. This indicated that yogurt bacteria did not affect significantly ($p< 0.05$) the composition of green and black teas phenolic compounds [69]. This was in agreement with Najgebauer-Lejko, [70] who found the concentrations of green tea infusion (5%, 10% or 15%) did not influence the viability of *S. thermophilus* and *B. animalis* ssp. *lactis* BB-12 in yogurt during 21 days of storage. However, the presence of green tea maintained the viability of bifidobacteria in yogurt at the average above 7 log cfu/g for extra 2 weeks compared to plain yogurt. On the other hand, Michael et al. [71] reported that the count of *L. bulgaricus* decreased below the recommended concentration of 6 log cfu/ml in yogurt after 2 weeks of storage. However, the presence of plant extract (0.5% and 1%; Cegemett® Fresh) increased the viability of this bacteria to more than 2 folds (> 6 log cfu/ml) until 4 weeks of storage which could related to prebiotics or sodium acetate existing in plant extract. The addition of plant extract (0.5%) did not adversely affect the viability of *S. thermophilus* during 50 days of storage [71]. Another study found that the addition of plant extract (garlic or cinnamon) in bio-yogurt did not affect the viability of *Lactobacillus* spp and *S. thermophilus* during 21 days of storage [72]. However, *B. bifidum* increased significantly ($p<0.05$) in the presence of these plant extracts as compared to the absence over 21 days of storage [73]. This meant that bacteria may behave differently from each other in the presence of phenolic compounds [63].

do Espírito Santo et al. [74] reported an increased ($p<0.05$) in the counts of *L. delbrueckii* subsp. *bulgaricus* (from 5.0 to 9.2 Log cfu/ml) in skim milk yogurt co-fermented by *L. acidophilus* L10 and the addition of fruit fibers such as apple or banana (1%) had no inhibitory effect on the viability of *L. delbrueckii* subsp. *bulgaricus*. Yet, in some cases as in yogurt co-fermented by the *B. animalis* subsp. *lactis* HN019, the presence of fibers from apple or banana have stimulated *L. delbrueckii* subsp. *bulgaricus* growth compared to the absence. This could be resulted of symbiotic relationship between apple or banana fibers and *B. lactis* HN019 that lead to enhance the viability of *L. delbrueckii* subsp. *bulgaricus*. In general, the presence of either apple or banana fibers showed an increase in the numbers of probiotics (*L. acidophilus* L10 *and B. animalis* subsp. *lactis* BL04, HN019 and B94) by no less than 1 Log cfu/ml compared to the absence. This could be related to their high contents of pectins and fructooligosaccharides that have prebiotic effect to enhance the bacteria growth [75,76].

Recently, Buriti et al., [77] studied the fermented whey-based goat milk and goat cheese beverages prepared using probiotic culture (*B. animalis* subsp. *lactis* BB-12, *L. rhamnosus* Lr-32 and *S. thermophilus* TA-40) with added guava or soursop pulps and with or without addition of partially hydrolysed galactomannan (PHGM) from Caesalpinia pulcherrima seeds. It was observed that both *B. animalis* and *L. rhamnosus* maintained good viability in the presence of either guava or soursop pulps. Although, including dietary fiber ingredients into food during processing has been widely used to increase the viability of probiotic during storage of products [74] however, *B. animalis* and *L. rhamnosus* showed inability to metabolize the PHGM, since no significant difference ($p>0.05$) between with and without PHGM. Similarly, Buriti et al. [78] found that PHGM was not fermented under *in vitro* conditions by the same probiotic strains. Oleuropein is a bioactive natural product from olives with variety of health beneficial properties. Zoidou et al. [79] detected that inclusion of oleuropein into yogurt during fermentation did not either metabolize by LAB or inhibit their growth and its remained stable in the final products.

7. Ice cream

Ice cream is a frozen dairy product produced from a combination of served ingredients other than milk. The composition of ice cream varies depending upon the ingredients used in its preparation. In many countries, the percentage composition of a good ice cream is 11–12% milk fat, 10–12% milk non-fat solids (MSNF), 12% sugar, 5% corn syrup solids, 0.3% stabilisers-emulsifiers [80].

Ice cream is a delicious and nutritious frozen dairy dessert with high calorie food value [81] and 82 g provides approximately 200 calories, 3.99g protein, 0.31g calcium, 0.10g phosphorus, 0.14mg iron, 548 IU vitamin A, 0.038mg thiamine and 0.23mg riboflavin [82]. Ice cream has nutritional properties but owns no therapeutic value [83]. Recently, the increasing demand from consumers for healthier and functional food has led to produce ice cream containing special ingredients with recognized nutritional and physiological properties such as dietary fibers [84], probiotics [85,86], lactic acid bacteria [87], prebiotics [58,88] alternative sweeteners [89], low glycemic index sweeteners [90] and natural antioxidants [55].

The main ingredient of ice cream is cow milk and this unfortunately may make dairy ice cream off limits to many consumers who suffer from lactose intolerance. Thus, replacing cow's milk with vegetables milk in general would help address two nutritional issues related to cow's milk: lactose intolerance and cholesterol content. Several researchers have used vegetable milk such as soy and coconut milk to produce probiotic ice cream with nutritional and theraputical properties [91-94]. Other studies found that the addition of plant ingredients such as watermelon seeds, ginger extract and black sesame could increase the overall acceptability of ice cream as well as enrich it with antioxidant activity [93,95,96].

Consumption ice cream containing probiotic strains could reduce bacteria levels in the mouth responsible for tooth decay [97]. Singh et al. [98] reported that consumption of probiotic ice-cream containing *B. lactis* BB12 and *L. acidophilus* La5 was associated with significant reduction in the levels of *Streptococcus mutans* in salivary of school children with no significant effect on lactobacilli levels. The pH and coliform counts of human faces of volunteers fed with synbiotic ice cream were significantly reduced ($p<0.01$) after two weeks of ingestion [99]. The pH reduction may be attributed to the production of short chain fatty acids by the colonic microbiota and probiotic bacteria [100,101]. In addition, consumption of ice cream containing probiotics such as *L. acidophilus* increased the faecal lactobacillus counts during 15 days of ingestion [99]. Ice cream prepared with probiotic culture such as *L. acidophilus* LA-5, *B. lactis* BB-12 and *Propionibacterium jensenii* 702 had a significant influence on the gastrointestinal tolerance (*in vitro*) after exposure to both highly acidic conditions (pH 2.0) and 0.3% bile [61]. This indicated that probiotic ice cream could improve the balance of the intestinal microflora of the host upon consumption [7,8] followed by immune system development [9].

8. Viability of Probiotic in Ice Cream

The growth and viability of probiotic bacteria are influenced by the temperature of the cultures medium. The effectiveness of probiotics ice cream consumption on consumer's health is associated with bacteria viability. Therefore, it is importance not only to reduce cell death during the freezing process but also to maintaining stability of bacteria during storage. Since ice cream is a whipped product, incorporation of large amounts of air into the mix resulting in oxygen toxicity, one of the most important factors of bacteria cell death. The viable counts of *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 in probiotic ice cream found to be significantly lower after freezing compared to prior to freezing [102]. The decline in bacterial counts in ice cream after freezing may occur due to the freeze injury of cells leading to the death of these cells. However, the mechanical stresses of the mixing and freezing process may have caused a further reduction in bacterial cells counts.

During ice cream freezing process, the probiotics can be lethally injured by damaging cell walls or rupturing their membranes because of the ice formed in the environment or inside the cell [103]. The rate of dehydratation of bacterial cells depends on the absorbency of the cell membrane and the surface area in relation to its volume. Thus, increase freezing rates may cause small ice crystals size with less damaging effects towards bacterial cells [103]. Rapid freezing of the ice cream mix obtained after inoculating with the probiotics contributes to maintain their stability in the recommended therapeutic doses. Some studies reported that the viability of the probiotic bacteria in ice cream after freezing is an important parameter to ensure compliance to the food industry standards and to meet consumer expectation [42,104].

Da Silva et al. [105] study the viability of *B. animalis* in goat's milk ice cream during storage. It was found that *B. animalis* decreased about 1 log and registered a rate of survival (84.3%) approximately 7 cfu/g during the first 24 hours of frozen storage. The viable cells counts of these bacteria were decreased about 1.26 log cycles with 84.7% survival rate during 120 days of frozen storage. This means that *B. animalis* had ability to maintain satisfactory viability in goat's milk ice cream during frozen storage (≥ 6.5 log cfu/g). The viability of novel probiotic *Propionibacterium jensenii* 702 included with *L. acidophilus* (La- 5), *B. animalis* subsp. *lactis* BB-12 in ice cream made from goat's milk was examined by Ranadheeraa et al. [102]. The viable counts of probiotic were found to be significantly lower by 56.14% for *L. acidophilus* and 66.46% for *B. lactis* after freezing whereas *P. jensenii* showed higher survival rate with 88.72%. This suggested that *P. jensenii* 702 may have mechanisms allowing survival during freezing which are not possessed by *L. acidophilus* and *B. lactis* [102]. These mechanisms may include ability of *P. jensenii* to dehydrate rapidly and thus decrease the formation of intracellular ice crystals that can damage cytoplasmic membranes and lead to cell death [106]. Anyhow, the final product of probiotic ice cream made from goat's milk found to be able to maintain satisfactory viability (10^7 to 10^8 cfu/g) over 52 weeks of the storage [102]. A mixture of human-derived probiotic strains of *L. acidophilus*, *L. agilis* and *L. rhamnosus* was used in ice cream manufacture [107]. The study stated that the viable cells counts of these bacteria remained constant in ice cream during 6 months of storage without any major loss of bacterial cells between in presence and in absence of sweeteners (sucrose or aspartame).

Previous study reported that *L. johnsonii* La1 and *L. rhamnosus* GG showed high survival rate in retail-manufactured ice cream with no decrease in the population inoculated initially (7 log cfu/g and 8 log cfu/g respectively) during storage up to 8 months for *L. johnsonii* and 1 year for *L. rhamnosus* [86,108]. Moreover, the sugar level (15% and 22% w/v), fat content (5% and 10% w/v) and even different storage temperatures (-16 °C and -28 °C) did not affect significantly on the viability of both probiotic bacteria [86,108]. The effects of inulin and different sugar levels on survival of probiotic bacteria in ice-cream were investigated by Akın et al. [85]. Ice cream produced by adding 10% w/w of fermented milk with commercial freeze-dried mixed probiotic culture consisting of *S. thermophilus*, *L. bulgaricus*, *L. acidophilus* LA-14 and *B. lactis* BL-01 to ice cream mix with different concentrations of sugar (15%, 18% and 21%; w/w) and inulin (1% and 2%). The results showed that ice cream content 18% sugar had the highest viable cells counts of bacteria. Yogurt bacteria in ice cream showed viability above 10^7 and 10^6 cfu/g for *S. thermophilus* and *L. bulgaricus* respectively during 90 days of storage. Moreover, the addition of inulin did not affect significantly on numbers of *S. thermophilus* or *L. bulgaricus*. However, the viability of *L. acidophilus* and *B. lactis* in ice cream found to increase from 10^5 cfu/g to 10^6 cfu/g after addition of 2% of inulin [85]. Likewise, Akalin and Erişir, [88] indicated that the survival of *L. acidophilus* La-5 and *B. animalis* BB-12 can be improved significantly ($p<0.05$) by addition of oligofructose in low-fat ice cream stored at -18°C for 90 days. *B. animalis* BB-12 maintained a minimum level of 10^6 cfu/g in only ice cream with oligofructose during storage period. Recently, Leandro et al. [87] reported that using of inulin to replace fat partially or totally in ice cream does not affect the viability of *L. delbrueckii* UFV H2b20 after processing and during storage. However, *L. delbrueckii* UFV H2b20 found to be differing from *L. acidophilus* La-5 and *B. animalis* BB-

12 which exhibited stability after freezing process and after 40 days of storage at -16 °C [88]. This suggested being associated with low overrun presented by the ice cream formulations. Similar observation has been demonstrated for *L. rhamnosus* [109]. Another study stated that incorporating fructo-oligosaccharides into probiotic ice cream significantly increased (p<0.01) survival of *L. acidophilus* and *Saccharomyces boulardii* during two weeks of freezing storage [83]. Non fermented probiotic ice cream made from vegetable milk (soy or coconut milk) improved the growth and viability of *B. lactis* and *L. acidophilus* during 30 days of storage at -20°C [94]. Furthermore, the study indicated that the survival of both probiotics was higher in soy milk ice cream than coconut milk ice cream which probably due to soy milk proteins that provide physical protection against freezing damage through encapsulating probiotics with stable network looks like a gel structure [110].

Several studies on survival of probiotic in ice cream during freeze storage have focused on the protective effects of encapsulation. Survival of free and microencapsulated *L. casei* (Lc-01) and *B. animalis* (BB-12) in symbiotic ice cream containing resistant starch as a prebiotic substance was studied [111]. The viable cells counts of free *L. casei* (Lc-01) and *B. animalis* (BB-12) in ice cream showed a decreased by 3.4 and 2.9 log respectively after 6 months of storage. However, encapsulated *L. casei* and *B. animalis* showed reduction by only 1.4 and 0.7 log throughout the storage period. Ice cream prepared by using encapsulated *L. casei* and *B. animalis* maintained viability of probiotic between 10^8 and 10^9 cfu/g overall shelf life. This indicated that encapsulation can significantly maintain high viability of probiotic bacteria in ice cream over storage. The observation is in line with Shah and Ravula, [112] who noted an improvement in counts of microencapsulated *L. acidophilus* MJLA1 and *Bifidobacterium* spp. BDBB2 compared to free cells in frozen fermented dairy dessert during 12 weeks of storage. Sahitya et al. [113] revealed that encapsulated *L. helveticus* 194 and *B. bifidum* 231 showed significantly (p<0.05) higher log counts (7.96 and 8.06 log10 cfu/g respectively) than non-encapsulated bacteria (6.06 and 6.33 log10 cfu/g respectively) at the end of 90 days of storage. In addition, co-encapsulated *L. helveticus* 194 and *B. bifidum* 231 along with prebiotics (3% Fructooligosaccharides) increased probiotic viability during storage at -20°C (Sahitya et al., 2013). Lately, Karthikeyan et al. [114]. evaluated the survivability of *L. acidophilus* (LA-5) and *L. casei* (NCDC-298) in ice cream using microencapsulation technique. Unencapsulated free *L. acidophilus* (LA-5) and *L. casei* (NCDC-298) showed about 3 log reduction over 180 days of storage at - 23°C with final cells counts of 6 log cfu/g and 7 log cfu/g respectively. However, microencapsulated improved the viability of *L. acidophilus* (LA-5) with only one log reduction during the entire shelf life and final bacteria counts of 8 log cfu/g whereas microencapsulated *L. casei* (NCDC-298) remained constant over storage with about 9 log cfu/g. Similar behavior has been displayed by *B. Lactis* (BB-12) with 30% increase in their viability in ice cream after microencapsulated with calcium alginate and whey protein for 6 months of storage [115].

9. Cheese

Cheese is a kind of fermented milk-based food product. It can also be regarded as a consolidated curd of milk solids in which milk fat is entrapped by coagulated casein [116]. The Food and Agriculture Organization of the United Nations (FAO) defines cheese as "the fresh or matured product obtained by the drainage (of liquid) after the coagulation of milk, cream, skimmed or partly skimmed milk, buttermilk or a combination thereof" [117]. Cheese contains, in a concentrated form, many of cow milk's nutrients and provided many essential nutrients such as protein and calcium; it also contains phosphorus, fat zinc, vitamin A, riboflavin and vitamin B12. Several bifidobacteria strains have been successfully incorporated into cheeses [118,119]. The addition of probiotic bacteria does not generally affect the gross chemical composition of cheese (i.e. salt, protein, fat and moisture) and pH [12;122]. Similarly, the primary proteolysis in cheese not influenced by added of probiotic cultures which in many cheeses occurred as a result of activity of the coagulant agent (except for high cook cheeses) and to a minor range by plasmin and subsequently residual coagulant and enzymes from the starter microflora [123]. However, addition of probiotic in cheese reported to effect on the changes of secondary proteolysis and the increases in free amino acid content as well as free fatty acid profile of cheese which directly contribute to cheese characteristics [120,124,125]. Most cheeses containing probiotic lactobacilli and bifidobacteria which have high lactic acid and acetic acid content due to lactose fermentation [120-122,125]. Bifidobacteria produce acetic and lactic acid in a ratio of 2:3 whereas lactobacilli produce lesser acetic compared to bifidobacteria [126]. Probiotic cheese provided an opportunity for lactose intolerant individuals due to a complete lactose hydrolysis that observed in several cheeses such as Crescenza, Canestrato Pugliese and Cheddar-like cheeses [127-129].

Probiotic cheese is believed to reduce the risk of heart disease and certain cancers [130,131]. Conjugated linoleic acid (CLA) is found in cheese, and recent scientific research supports potential roles for CLA isomers in reducing the risk of certain cancers and heart disease, enhancing immune function and regulating body weight/ body fat distribution [132]. Cheese with *L. rhamnosus* HN001 and *L. acidophilus* NCFM found to be beneficial in improving the immune response of healthy elderly subjects [133]. Probiotic fresh cheese allows *B. bifidum, L. acidophilus* and *L. paracasei* to exert significant immunomodulating effects in the gut [134]. The pure cultures of *B. bifidum* and *L. paracasei* were identified in small intestine of mice fed with probiotic fresh cheese whereas *L. acidophilus* was mainly identified in the large intestine [134].

Probiotic cheese reduces the risk of dental caries (decay) which usually results from the breakdown of tooth enamel by acids produced during the fermentation of sugars and starches by the plaque bacteria [135]. The short-term consumption of probiotic cheese containing *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC 705 reduced caries-associated salivary microbial counts such as *Streptococcus mutans* by 20% and salivary yeast by 27% in young adults [136]. The protective effect of cheese against dental caries may also be explained by an antibacterial effect of components produced during metabolic activities of probiotic bacteria in cheese (e.g., fatty acids, organic acid, peptides etc.).

10. Viability of Probiotic in Cheese

Probiotic bacteria can be included into cheese during manufacture in two ways either as a starter (depended on the ability to produce adequate lactic acid in milk) or as adjunct to the starter culture which is more favourable option to incorporate probiotic with the starter bacteria during cheese making. A few approaches have been applied to improve the survival of probiotic in cheeses one of them is the use of different combination of starter and probiotic [131]. The development of probiotic cheeses can be very strain dependent as many of the probiotic strains showed poor performance in the cheese environment. Strain selection plays a key role in successful development of probiotic cheese. In addition, processing conditions, cooking procedure, the aerobic environment, temperatures of ripening and storage are affecting viability of probiotic bacteria a well as the concentration of these bacteria in the final product provides a therapeutic dose to consumers [131]. *Lactobacillus acidophilus* (La-5) is a probiotic bacterium that important to be survived in cheeses during production and storage of probiotic cheeses. In order to exert the beneficial effects of probiotic foods at the minimum probiotic therapeutic daily dose intake 100 g of a food product containing 6 or up 7 log cfu/g [137]. The viability of probiotic culture of *L. acidophilus* fund to be above 6.00 log cfu/g during storage in minas fresh cheese, festivo cheese, white brined cheese, argentinian fresco cheese, semi-hard argentinean fresco cheese, petit suisse cheese and Tallaga cheese [121,138-143].

Tharmaraj and Shah, [144] found the best combination of probiotic bacteria can be used in cheese-based dips when combined *L. acidophilus*, *B. animalis* and *L. paracasei* subsp. *paracaseiin* together (inoculation at 9 log/g). The *L. acidophilus* and *B. animalis* showed a high level of population required for health benefit through 10 weeks of storage period. However, the presence of *L. rhamnosus* and *P. freudenreichii* subsp. *shermanii* in cheese-based dips with the above mentioned combination had no significant effect of the bacteria in the combination and can be inoculated at level of 7 log to keep the viability above 6 logs during 10 week of storage. The viability of *L. casei*, *L. rhamnosus* GG or probiotic mix YO-MIX™ 205, including *L. bulgaricus*, *L. acidophilus*, *Bifidobacterium* spp. and *S. thermophilus* added to cottage cheese during storage was observed by Abadía-García et al. [145]. All the added probiotic bacteria persisted viable in cottage cheese throughout 28 days of storage. Cottage cheese including *L. casei* or YO-MIX ™205 showed higher viable cell counts of 8 $\log_{10}$ cfu/g over the last 2 weeks of storage at 8 °C. Conversely, *L. rhamnosus* GG remained constant at levels of 6 $\log_{10}$ cfu/g over the whole storage period [145]. Six batches of Cheddar cheeses inoculated with different probiotic bacteria used as an adjunct including *B. longum* 1941, *B. animalis* subsp. *lactis*

B94, *L. casei* 279, *L. casei* L26, *L. acidophilus* 4962 or *L. cidophilus* L10 [118]. The viability of probiotic in all cheese batches were remained at the level of 8-9 log_{10} cfu/g at the end of the production process. The amounts of starter lactococci in cheese batches inoculated with *B. animalis* B94, *L. casei* L26 or *L. acidophilus* were significantly reduced ($p < 0.05$) by the ripening temperature at 8 °C compared to those at 4 °C after 24 weeks. However, the probiotic cells in cheeses with different strains of probiotic were not significantly ($p > 0.05$) different during the ripening period (24 weeks) and ripening temperature (4 °C and 8 ° C). [146] found that the combination of *L. paracasei* A13 with probiotic (*B. bifidum* A1, *L. acidophilus* A3) and starter (*Lactococcus lactis* A6 and *S. thermophilus* A4) in Argentinian fresh cheese improved viability of *L. paracasei* A13 by approximately half log order during the production process at 43 °C and another half log order during the first two week of storage at 5 °C. In addition, increase storage temperature to 12°C (temperatures usually found in retail display cabinets in supermarkets) had positive effect on the growth of *L. paracasei* A13 by almost 2 log orders from day 30 until day 60 [146].

The impact of two different techniques (pre-incubation step or directly to the vat) for the inoculation of probiotics mixture (*L. acidophilus*, *L. paracasei* and *B. lactis*) on the viability of these probiotics during semi-hard cheese ripening for 60 days was investigated by [119]. They found no significant differences in the counts of each probiotic strain at the end of the ripening regardless their addition as lyophilised or after pre-incubation. In addition, *L. paracasei* strain registered the highest cell counts ~10^9 cfu/g followed by *L. acidophilus* and *B. lactis* with cell concentration of 10^8 cfu/g/ and 10^7 cfu/g respectively [119]. This study was in line with previous study conducted by Bergamini et al. [121] who found no significant differences between using the two techniques in inoculation of probiotic bacteria in semi-hard Argentinean cheese (freeze-dried powder or after pre-incubation). Lyophilized or freeze-dried powder technique is a more effective process because it is easier, cheeses are not over acidified and the probiotic population at the end of ripening is relatively similar to that in pre-incubation in substrate composed of milk [119,122]. Recent study found a new invention process consisting in an edible sodium alginate coating as carrier of probiotic (*L. rhamnosus*) and prebiotic (fructooligosaccharides) which was effective in manufacture functional Fiordilatte cheese [147]. Research results indicated that the a consumption of 100 g of coated Fiordilatte cheese provide a daily dose of probiotics equal to 10^9 cfu/100g which recommended for health purpose. However, the functional acceptability limits for the coated Fiordilatte cheese with probiotics and prebiotics were 8 days at 4 °C, 6 days at 9 °C and 5 days at 14 °C [147].

Besides the acceptable probiotic viable counts, the behavior of probiotics in presence of prebiotics in cheese have been widely studied [147-149]. The addition of both inulin and oligofructose combined in petit-suisse cheese showed satisfactory probiotic viable counts of *L. acidophilus* and *B. animalis* subsp. *lactis* during 30 days of storage [148]. This performance has not observed in other studies where inulin had no significant effect on growth and survival of *L. paracasei* in a synbiotic fresh cream cheese [149]. Likewise, the presence of inulin or a mixture of inulin and fructooligosaccharides (50:50) in the synbiotic cheeses was not affected the viability of *L. casei* 01 and *B. lactis* B94 during 60 days of ripening period [150]. Therefore, the improvement of probiotic cheeses in presence of prebiotics such as inulin, oligofructose and fructooligosaccharides could be very strain and cheese type dependent. In addition, the populations of *L. acidophilus* in Caprine Coalho cheese naturally enriched with conjugated linoleic acid (CLA) were no statically significant ($p>0.01$) compared to Caprine Coalho cheese prepared without CLA-enhanced milk during 60 days of storage [149]. However, the stability of CLA content (isomer C18:2 cis-9, trans-11) in Caprine Coalho cheese was observed during the ripening period. This could provide healthier fatty acid profile, offering an increased CLA, oleic and linoleic acid levels along with a lower content of total saturated fat [149].

11. Conclusion and Recommendations

Dairy food is a promising food matrix for probiotics. Generally, probiotic yogurt developed for the market considered to be competitive as compared with probiotic cheese or ice cream. In addition, a number of studies regarding to including plant materials to probiotic yogurt have been successfully established to increase the viability of probiotic during production and storage. However, such an approach has not developed sufficiently in probiotic cheese or ice cream which could have a significant impact on probiotic survival. The interaction between phenolic compounds from plants extracts and probiotic bacteria has not been fully understood yet. The bacterial species and strain in addition to chemical structure and concentration of the polyphenols play a significant role in sensitivity of probiotic to the phenolic compounds. Furthermore, strain selection and possible process modifications should be carefully assessed to promote probiotic cells in dairy food during manufacture and storage to ensure health benefits can be delivered to consumers on daily consumption. More additional studies might be needed to evaluate *in vivo* therapeutic properties of probiotic yogurt, ice cream and cheese.

Table 1: Examples of probiotic bacteria used in probiotics dairy products.

Lactobacillus species	Bifidobacterium species	Other
L. acidophilus	*B. bifidum*	*Streptococcus thermophilus*
L. casei	*B. longum*	*Propionibacterium jensenii*
L. helveticus	*B. lactis*	*Propionibacterium freudenreichii subsp. shermanii*
L. plantarum	*B. adolescentis*	*Lactococcus lactis ssp. lactis*
L. rhamnosus	*B. infantis*	*Enterococcus faecium*
L. agilis	*B. breve*	*Lactococcus lactis ssp. cremoris*
L. johnsonii	*B. animalis*	*Leuconostoc mesenteroides ssp. dextranicum*
L. paracasei		*Pediococcus acidilactici*
L. gasseri		

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