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Ice Cream and Frozen Yoghurt - A Suitable Carrier for Probiotics: A Review

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ABSTRACT

Nowadays there has been a significant interest in the development of innovative functional food products conferring customized benefits to the consumers *viz.,* physical and mental well-being, dental health, gastro-intestinal functions, *etc*. Among the dairy products with live cultures, probiotic ice cream and fermented frozen desserts such as frozen yoghurt are the emerging functional foods. Ice creams and frozen yoghurt are food products showing potential for use as probiotic vehicles and are highly popular with the consumers. The problem to be tackled relates to the loss of viability of probiotic cells in frozen dairy desserts which can occur during product formulation, processing, freezing and storage. The development of probiotic frozen dairy desserts containing live probiotic bacteria necessitates certain technological interventions. The means used to attain higher survivability of probiotic cells in such frozen products include use of selected probiotic strains, use of prebiotics along with probiotics, encapsulation of probiotic cells, use of cryoprotectants, *etc*.

Key words: Cell viability, Frozen yoghurt, Ice cream, Prebiotic, Probiotic, Sensory quality.

Dairy products represent one of the biggest market segments among functional foods as they have been proposed to be the most promising vehicles for the delivery of functional ingredients such as probiotic, prebiotics, omega fats, proteins, vitamins, minerals, phytochemicals, *etc*. (Akin and Ozcan, 2017). The dairy industry has put probiotic cultures in the forefront for the development of newer functional products. Yoghurts and fermented milks are the main vehicles for probiotic cultures (Champagne and Gardner, 2005), while ice cream is a food product having good potential to act as a carrier for delivery of probiotic bacteria to the consumers, especially due to the frozen storage conditions.

Ice cream is a complex multiphase system comprising of ice crystals, air cells and fat globules embedded in a highly viscous, freeze-concentrated matrix phase (Eisner *et al*., 2005). Ice cream is a highly nutritive product. There are studies demonstrating the suitability of ice cream for delivering probiotics in the human diet (Hekmat and Mc Mahon, 1992; Alamprese *et al*., 2005; Akin *et al.,* 2007; Parussolo *et al*., 2017). However, these target microorganisms, depending on their characteristics, could be unstable in these products owing to mechanical, freezing, oxidative and/ or osmotic stresses.

Frozen yoghurt is a fermented frozen dairy product that mimics the physical characteristics of ice cream as well as the sensory and nutritional traits of fermented milk product. Commercial frozen yoghurt is reported to contain > 10^7 cfu/g of lactic acid bacteria (LAB) (Soukoulis and Tzia, 2008). Frozen yoghurt is usually prepared utilizing two species *i.e*., *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* (Davidson *et al.,* 2000). Several researchers (Hekmat and Mc Mahon, 1992; Hagen and Narvhus, 1999) have tried to produce probiotic frozen yogurt through introduction of probiotic bacteria (*L. acidophilus* and/or *B. bifidum*) in such food.

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The aim of the present paper is to review the technological parameters involved in the production of probiotic ice cream and frozen yoghurt type products. In manufacturing and storage of such frozen products, several hurdles are encountered during processing which leads to decline in the viability of probiotic cells. These have been counteracted adequately by the researches focused by the scientists on these aspects.

Probiotic concepts

The word probiotic means 'for life' (Fuller, 1989). Probiotic is used to refer to cultures of live microorganisms which, when administered to humans, improve the properties of indigenous microbiota (Margoles and Garcia, 2003). They are live microbial food ingredients that are beneficial to human health (Clancy, 2003).

Probiotic foods are considered one of the fastest growing categories of functional foods (Chavez-Tapia *et al*. 2015). Fermented foods such as yogurt and cheese are considered popular food delivery systems for probiotic cultures (Karimi *et al*., 2011; Araujo *et al.,* 2012). Since yogurt is one component of frozen yogurt, with the coupling of delicate flavor of fruit, it serves as a very apt medium for probiotics too. *Lactobacillus* and *Bifidobacterium* are the most common species of bacteria used as probiotics for the production of valued dairy products (Abdelazez *et al*., 2017).

Types of probiotic microbes

The basic concept behind probiotics is to restore the depleted ecosystem of the human intestine with new, healthful bacteria. A number of genera of bacteria and some specific yeasts are used as probiotics, including *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium* and *Enterococcus*; main species having probiotic potential are *L. acidophilus*, *Bifidobacterium* spp. and *L. casei* (Fuller, 1997). The probiotic yeast is *Saccharomyces boulardii* (Lourens-Hattingh and Viljoen, 2001). Probiotic bacteria with desirable properties and well-documented clinical effects include *L. johnsonii* La1, *L. rhamnosus* GG (ATCC 53103), *L. casei shirota*, *L. acidophilus* NCFB 1478, *B. animalis* Bb12 and *L. reuteri* (Shah, 2004).

Fermented foods containing live probiotic cultures

In Japan, a standard developed by the Fermented Milks and Lactic Acid Bacteria Beverages Association demand a minimum of 10^7 cfu/mL of probiotic microorganisms at the end of the product's shelf-life (Ishibashi and Shimamura, 1993). Food products having a longer shelf-life such as probiotic ice cream and frozen yoghurt (Mohammadi *et al.* 2011; Tungrugsasut *et al.* 2012) have been dealt lately.

Application of probiotic bacteria in dairy foods

There is evidence that food matrices play an important role in the beneficial health effects of probiotics on the host (Espirito Santo *et al*., 2011). Fermented foods, particularly dairy foods, are commonly used as probiotic carriers. Fermentation of dairy food has several advantages *viz.,* preserves food, improves nutritional value and sensory quality (Gadaga *et al*., 1999).

Bifidobacteria have been incorporated into dairy products such as yogurt (Oliveira *et al*., 2011), cheese (Fritzen-Freire *et al*., 2010), ice cream (Akin *et al*., 2007) and frozen yogurt (Davidson *et al*., 2000).

Therapeutic benefits of probiotic microorganisms

Health benefits

Certain members of *Lactobacillus* and *Bifidobacterium* spp. are reported to decrease the levels of carcinogenetic enzymes produced by colonic flora through normalization of intestinal permeability, microflora balance, production of organic acids and enhancing the host's immune system (Kumar *et al*., 2010). Food products containing probiotic bacteria could possibly contribute to prevention of coronary heart disease (CHD) by reducing the serum cholesterol levels as well as to blood pressure control (Sanders, 2003).

Probiotics such as *L. rhamnosus* GG*, L. reuteri, L. casei shirota and B. animalis* Bb12 have proved to shorten the duration of acute rotavirus diarrhea in clinical trials (Szajewska and Mrukowicz, 2001). Consumption of yoghurt by lactose intolerant individuals do not pose any difficulty to them owing to adequate β-galactosidase activity and fermentation (Gowri *et al.,* 2016).

Allergy

Probiotics are able to treat allergies resulting in decreased inflammation, stabilizing the immune system and strengthening gut lining. Probiotics modify the structure of antigens, reduce their immunogenicity and intestinal permeability (Gowri *et al.* 2016). *Lactobacillus* GG and L. *rhamnosus* GG are reported to alleviate the symptoms of food allergies (Manoj *et al.* 2012).

Inflammatory bowel disease

Probiotics are used to control inflammatory bowel diseases such as ulcerative colitis, Crohn's disease and Pouchitis. Potential mechanisms include suppression of growth or epithelial binding and invasion by pathogenic bacteria, antimicrobial substances, epithelial barrier function and immune regulation (Momir *et al*., 2014).

Hypertension

Probiotics and their metabolic products can improve blood pressure through mechanisms improving total cholesterol and low-density lipoprotein cholesterol levels (Guo *et al.* 2011). *L. helveticus, L. rhamnosus* GG*, L. casei, L. acidophilus, B. breve* and *B. longum* are implicated in exerting anti-hypertensive effects (Golnaz *et al.* 2017).

Cholesterol reduction

The cholesterol levels can be cut down directly or indirectly using probiotics. Direct mechanism involves inhibition of de novo synthesis or decrease in the intestinal absorption of dietary cholesterol, while indirect mechanism involves deconjugation of the cholesterol to bile acids. *B. animalis* subsp. *lactis* MB 202/DSMZ 23733, *B. bifidum* and *B. breve* exerted such effects (Bordoni *et al.* 2013).

Technological hurdles in producing probiotic frozen dairy foods

The important criteria for manufacture of probiotic frozen foods are to maintain the viability of probiotic cultures till the end of their shelf life. Superior survival rate of probiotics in products have been obtained using techniques such as culturing of ice cream mix (Hekmat and Mc Mahon, 1992; Davidson *et al.* 2000; Akin 2005), using non-fermented ice cream mix (Alamprese *et al.* 2002; Haynes and Playne, 2002), or adding fermented mix to regular ice cream mix (Hagen and Narvhus, 1999).

At each stage of processing, probiotic cells encounter various stress factors, including detrimental conditions in the product formula *viz.*, pH, titratable acidity and sugar content; other factors include freezing injuries, oxygen toxicity and mechanical stress. Temperature fluctuations during frozen storage of such products should be avoided. The factors affecting the viability of probiotic bacteria in ice cream/frozen yoghurt from formulation to consumption are discussed herein.

Effect of formulation of ice cream mix/frozen yogurt mix

Sugar exerted complex effects on the viability of probiotics in frozen products. The sugar associated with ice cream and frozen yogurt is usually 12.5-18.0% and 15.0-21.0% respectively. The sugar content varied in the products depending on the flavoring matter used (Akin *et al.* 2007; Mohammadi *et al.,* 2011; Soodbakhsh *et al.,* 2012). Some probiotic organisms, *viz*., *Lb. reuteri* (Hagen and Narvhus, 1999) or *B. bifidum* (Ma, 1995) tend to produce a slightly acid flavor in probiotic food. Senanayake *et al.* (2013) reported that use of wood apple puree at level of 10.0% by weight as flavoring agent in frozen yogurt led to a marked reduction in the count of *L. acidophilus* LA 5 during the initial stage of frozen storage, subsequent reduction in count was gradual.

Sugar exceeding 15.0% might reduce probiotic survival because of osmotic stress and impact on freezing point depression (Jay *et al.,* 2005). Only selected strains, such as *L. johnsonii* La1 were able to survive the relatively high sugar (up to 22.0%) content in ice cream. Counts of 10^7 cfu/g for bifidobacteria in frozen yoghurt were maintained for 10 weeks (Kebary *et al*., 2004) or counts of *Lactobacillus johnsonii* La1 were maintained in ice cream for 8 months of frozen (-28°C) storage (Alamprese *et al*. 2002).

The pH and titratable acidity of probiotic products markedly influences the survival of probiotic bacteria in frozen dairy foods. The optimum pH for growth of *L. acidophilus* was 5.5-6.0, but for bifidobacteria it was 6.0-7.0. The pH of probiotic ice creams varied from 5.0-6.0, while ice cream had a pH nearing 6.5 (Hekmat and Mc Mahon, 1992). The titratable acidity of probiotic ice cream varied from 0.24 – 0.27% LA (Salem *et al.,* 2005). The fat content of ice cream did not have any adverse effect on the probiotic cells (Mohammadi *et al.,* 2011).

Effect of freezing process

During freezing, the probiotic cells can be lethally injured through damaged cell walls caused by the mechanical stresses of ice crystals, also by cold injuries and temperature shock to the cells in the extra/intracellular medium (Jay *et al.* 2005; Akin *et al.* 2007). The size of the ice crystals increases as the freezing rate decreases; larger intracellular ice crystals (≥ 50 µm) cause greater damage to the cells (Jay *et al.,* 2005). Only *Lactobacillus johnsonii* La1 could survive the relatively high sub-lethal injuries caused by freezing (Kebary *et al*. 2004). Rapid freezing of the ice cream mix after inoculating with the probiotic bacteria is recommended to preserve viability of cells.

Effect of overrun

Most of the probiotic microorganisms are facultative anaerobic. Besides freezing injuries to probiotic cells, the incorporation of air into the mix results in greater decrease in viable cell counts. Oxygen content in product and redox potential affects the viability of bifidobacteria during processing and storage. Most probiotic strains *viz., Lactobacillus* and *Bifidobacterium* genera are microaerophilic or anaerobic (Champagne and Gardner, 2005).

Effect of storage conditions of frozen product

During storage, variations in the survival rate of probiotic bacteria depend on the strain, product formulation, production technology, storage conditions (Hekmat and Mc Mahon, 1992; Hagen and Narvhus, 1999). Several studies have revealed that the probiotic cell count decreased markedly throughout storage.

Hekmat and Mc Mahon (1992) determined the survival of *L. acidophilus* and *B. bifidum* in probiotic ice cream. The counts of *L. acidophilus* and *B. bifidum* in fermented product immediately after freezing were 1.5×10^8 and 2.5×10^8 cfu/mL respectively. At the end of 17th week of storage at -29°C, the population of the respective cultures decreased to 4×10^6 and 1×10^7 cfu/mL respectively.

The viable counts of probiotic ice cream decreased by 2.23, 1.68, 1.54, 1.23 and 1.77 $\log_{10}$cfu/g for *L. acidophilus, B. bifidum, L. reuteri, L. gasseri* and *L. rhamnosus* respectively during 12 weeks of frozen (-26°C) storage; product was prepared mixing milk fermented with probiotics with ice cream mix (Salem *et al*., 2005). The freezing and mixing operations had a greater effect on culture viability than the storage of ice cream (Hagen and Narvhus 1999; Alamprese *et al.,* 2002; Akalin and Erisir, 2008).

Techniques to improve the viability of probiotic cells in frozen dairy foods

Since legal requirements dictates to have minimum number of probiotic cells count at the end of the product's shelf life, there is a need to involve technologies that can improve the rate of survival of probiotic cells till such period. Some of these factors are discussed herein.

Prebiotics

They can markedly improve the retention of probiotic viability (especially for bifidobacteria) in food products as well as in the gastrointestinal tract (Gibson *et al.,* 2004). The addition of prebiotics such as inulin markedly improves the viability of probiotic organisms (Crittenden *et al*., 2001).

Microencapsulation

It is a technique to preserve microbial cells in functional foods and delivery of viable bacteria to intestine (Anal and Singh, 2007). Encapsulation could improve the survival of probiotic bacteria in synbiotic ice cream (Homayouni *et al*., 2008).

Immobilization

Immobilizing micro-organisms by entrapment in gel beads (matrix gelation) is considered a method to preserve their viability. Such technique can extend shelf life, prevent exposure to oxygen and improve resistance to gastric and bile acids (Le-Tian *et al*., 2004).

Cryoprotectants

Their incorporation resulted in increased survival of bifidobacteria *i.e.* to tune of 88.5% (Kebary *et al.,* 2004) owing to reduced ice crystal formation by binding with water by such agents. (glycerol, malt extract).

Elimination of molecular oxygen

Freezing of dairy desserts involves incorporation of air to have desired overrun. Use of oxygen scavengers such as L-ascorbic acid and potent redox potential reducing agents *(viz.,* L-cysteine) along with barrier packaging material can reduce the amount of oxygen in dairy products (Miller *et al.* 2003). Media used for the enumeration of bifidobacteria often contain L-cysteine (0.5-0.1 g/100 mL) in order to improve the bacterial recovery.

Ice cream and frozen yoghurt as probiotic carrier

The growing interest of consumers in therapeutic products has led to the incorporation of probiotic cultures into ice cream (Cruz *et al*., 2009). During probiotic ice-cream production, the processing steps involved are required to be optimized, in order to have increased survival of the probiotic bacteria (Goff, 2008).

Among dairy products with live cultures, probiotic frozen dairy desserts are gaining popularity nowadays (Cruz *et al,* 2009; Akalin *et al*., 2018). It was feasible to produce frozen yoghurt using different ratios of fermented mixes *viz*., ice cream mixes inoculated with 4.0% of *L. acidophilus* and *B. bifidum* (Hekmat and Mc Mahon, 1992) and ice cream mixes inoculated with two-culture probiotic mix (*B. longum* and *L. acidophilus)* at level of 0.02% by weight of mix (Davidson *et al*., 2000).

Incorporation of probiotic cultures in ice cream

Incorporation of inulin at the rate of 2.0% in probiotic vanilla ice cream stimulated the growth of *L. acidophilus* and *B. lactis* with improved viability of cells. The probiotic ice-cream had *L. acidophilus* and *B. lactis* count of 8.79 and 9.0 log_{10}cfu/g respectively (Akin, 2005). The probiotic *L. rhamnosus* GG culture, added at level of 0.2 % in ice cream (5.0% fat, 22.0% sugar), survived up to 1 year at -28°C (Alamprese *et al*., 2005).

Salem *et al.* (2005) noted a decrease in the viable count in vanilla flavored probiotic ice cream (8.0% fat, 12.0% MSNF, 16.0% sugar prepared by mixing fermented milk with ice cream mix) by 2.23, 1.68, 1.54, 1.23 and 1.77 log cfu/g for *L. acidophilus*, *B. bifidum, L. reuteri, L. gasseri* and *L. rhamnosus* respectively during 12 weeks of frozen storage. Frozen yogurt samples prepared using *B. bifidum* Bb-12 (score of 90) and *L. reuteri* B-14171 had acceptable sensory scores (out of 100) vis-à-vis control frozen yogurt (*i.e.,* score of 90.0 and 89.0 vs. 94.0 for control).

Probiotic vanilla ice cream incorporated with inulin at 2.0% stimulated the growth of *L. acidophilus* and *B. lactis* and improved their viability. *S. salivarius* ssp. *thermophilus* was most stable in all samples of probiotic ice-cream with count >10^7 cfu/g throughout the storage period of 90 days (Akin *et al.,* 2007).

The ice cream prepared using combination of freeze-dried cultures of *L. acidophilus* and *B. bifidum* (1:1) at 1.0% level, added prior to freezing, had similar sensory acceptability to that of control ice cream (Vijayageetha *et al*., 2011).

Ahmadi *et al.* (2012) developed synbiotic yogurt-ice cream *via* incorporation of fructo-oligosaccharide (FOS 4.0 - 8.0%) and microencapsulated *L. acidophilus* la-5 using Ca-alginate as coating material. Supplementation of yoghurt-ice cream with FOS increased its overrun. Viable number of encapsulated *L. acidophilus* was decreased by < 1 log cycle at 60th day of frozen storage.

Parussolo *et al.* (2017) prepared synbiotic strawberry flavored ice cream using *L. acidophilus* NCFM culture (@ 0.06 and 0.13%) along with yacon flour (up to 3.0%); the probiotic count was ≥10^6 cfu/g at 150th day of storage. Hekmat and Mc Mahon (1992) prepared strawberry flavored probiotic ice cream using *L. acidophilus* and *B. bifidum* at level of 2.0% each; the counts were 1.5×10^8 cfu/mL for *L. acidophilus* and 2.5×10^8 cfu/mL for *B. bifidum*. At 3.5 months, the count decreased to 4×10^6 and 1×10^7 cfu/g for the respective cultures.

dos Santos Cruxen *et al.* (2017) developed fruit ice cream using pindo palm fruit (*Butia odorata*) pulp at level of 50.0%, supplemented with *B. lactis* (Bl-04). The viable probiotic count was > 10^6 cfu/g on 90th day of storage (-18°C).

Sengsaengthong and Oonsivilai (2019) reported that microencapsulation of *Lactobacillus* sp. 21C2-10 by an emulsion technique using maltodextrin and gelatin helped in improving the survival of cells (survival of 93.86% vs. 72.02% for free cells) during frozen storage.

Probiotic cultures for frozen yoghurt

Hussein and Aumara (2006) prepared low fat (4.0%) vanilla flavored frozen yoghurt using probiotic (*L. delbrueckii* subsp. *bulgaricus*, *S. salivarius* subsp. *thermophilus* and Bifidobacteria) along with yoghurt culture; roasted red sweet potato puree (31.20% TS) and pumpkin puree (10.30% TS) were used at level of 25.0% each. The counts of *L. bulgaricus*, *S. thermophilus* and *L. acidophilus* were higher by 0.5-0.7 log cfu/g in product containing pumpkin solids as compared to that having sweet potato. The glucose and fructose of pumpkin possibly promoted the growth of bacteria. Storage of frozen yogurt (using *L. acidophilus* and bifidobacteria cultures) for 8 weeks led to product having viable count ranging between 7.00 and 8.80 log_{10} cfu/g.

Probiotic frozen yoghurt (using Bifidobacterium) made from camel milk was associated with markedly higher viscosity (530.9 mPas) as compared to cow milk product (215.0 mPas); the former product had greater melting resistance, but had lower flavor scores as compared to cow milk (*i.e*., scores of 3.55 and 4.77 respectively for camel and cow milk products) product (Al-Saleh *et al.,* 2011).

Pinto *et al.* (2012) reported that microencapsulation of probiotic culture, especially Bifidobacteria using reconstituted skim milk and inulin (20.0% w/v) improved the stability of such cells (~ 90 days) in frozen yogurt. Control frozen yoghurt utilized 0.6% (v/v) of bacterial suspension of bifidobacteria, while experimental product involved 2.0% (w/v) of microcapsules.

Tungrugsasut *et al.* (2012) prepared frozen yogurt utilizing 3.0% w/w probiotic culture (*Lactobacillus* spp.); the survival rate of culture at 30th day was 34.0%. Use of probiotic culture *L. acidophilus* r2 in addition to yoghurt culture led to greater survival (≥ 10^6 cfu/g) of culture at 4 weeks frozen (-18°C) storage vs. *Lactobacillus acidophilus* r1 and *L. lactis* subsp. *lactis* r3 (Mahrous and Salam, 2014).

Soodbakhsh *et al.* (2012) prepared frozen yoghurt containing free and encapsulated (using 2.0% alginate beads) *L. casei* (Lc-01) and *B. lactis* (Bb-12). The viable cell number of *L. casei* and *B. lactis* (used in free state) in the freshly prepared product was 4.7×10^8 and 5×10^8 cfu/g respectively; such count decreased to 4.5×10^6 and 3.5×10^6 cfu/g, respectively at 150th day. The pertinent counts using encapsulated *L. casei* and *B. lactis* cells were 7.3×10^8 and 9.1×10^8 cfu/g respectively when fresh; the counts increased to 8.6×10^7 and 9.6×10^7 cfu/g respectively at 150th day.

Abdelazez *et al*. (2017) employed *B. adolescentis* ATCC 11550 and *B. infantis* ATCC 11551 along with yogurt culture to prepare frozen yogurt. The *B. adolescentis* count of product was 2.6×10^8 cfu/g when fresh, while the count decreased by 71.20% at 60th day. The count of fresh product prepared using *B. infantis* with yogurt culture was 2.73×10^8 cfu/g which declined in count by 76.80% at 60th day of storage.

Frozen yogurt was prepared using probiotic cultures (*i.e.*, *L. acidophilus* and *B. lactis*) @ 0.02% with yogurt culture. Inulin (2.0 to 6.0%) and glycerol (1.0 to 4.0%) were used as prebiotic and bulking agent and freezing point depressant respectively. Incorporation of inulin at 4.0 and 6.0% level favored the overrun of product; increase in overrun was by 3.0 and 5.0%; the hardness of product decreased by 7.0% and 11.00% respectively (Muzammil *et al.,* 2017). Use of 4.0% glycerol favoured viability of probiotic cells in product. The loss of viability of *L. acidophilus* and *B. lactis* was to the extent of 0.5 and 0.14 log cycles during freezing process, which further declined by 2.29 and 2.81 log cycles respectively at the end of 12 weeks storage (Muzammil and Rasko, 2018).

Frozen yogurt mixes (2.0, 4.0% milk fat) were formulated using ultrafiltered (UF) skim milk as the base material and fermented using *L. acidophilus*, *B. bifidum* and a mixed yogurt culture. The acid production in UF mixes progressed well, however, excessive viscosity was noted in mix when the acidity exceeded 0.40% LA. The probiotic culture was stable during 6 weeks of frozen storage (-29°C). The counts were 5.14, 5.72 and 6.62 $\log_{10}$cfu/g for mixed yoghurt culture, *L. acidophilus* and *B. bifidum* respectively (Ordonez *et al.,* 2000). Forbes *et al.* (1996) reported that frozen yogurt produced from UF skim milk retentate (20.0% TS) using probiotic cultures (*viz.*, *Bifidobacterium* and *Lactobacillus* spp.) had viable count 10^7 cfu/g, even at 6 months.

Hippophae rhamnoides L. berries were used as immobilization carrier for the probiotic *Lactobacillus casei* ATCC393. The immobilization was performed by mixing berries with *L. casei* biomass (10:4 w/w) in deproteinized cheese whey. The free and immobilized probiotic cells were used as adjunct with yogurt culture. The viability of the immobilized probiotic cells was maintained (> 10^7 cfu/g at 90 days; count was 9.98 log cfu/g when fresh) during 90 days storage (-18°C), while the viability of free cells decreased by ~10.0% (Terpou *et al*., 2019).

CONCLUSION

Amongst dairy foods, ice cream and frozen yoghurt showed great potential for use as vehicles for probiotic cultures to develop wellness products having therapeutic virtues. Probiotic ice cream and/or frozen yogurt should ideally be manufactured using probiotic culture strains that shows stability during the frozen storage of the product. The flavoring matter used in these frozen foods are able to counteract the lowering in sensory rating; alternate method is to use probiotic cultures in association with LAB. In order to develop such products, besides selection of cultures, inoculum concentration, means employed to retain viability of cells, appropriate stage of incorporation of culture, strict control over the processing protocols and transport and storage conditions needs to be considered.

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