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REVIEW ARTICLE

Probiotic ice cream: viability of probiotic bacteria and sensory properties

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Abstract Ice cream is a dairy product with good potential to act as a food carrier for probiotic bacteria. The incorporation of probiotic bacteria into ice cream is highly advantageous since, in addition to making a functional healthy food, ice cream in itself contains beneficial substances such as dairy raw materials, vitamins and minerals, and is consumed by the general population. Also, compared with fermented milks as a vehicle, ice cream supports considerably greater viability of probiotic strains during production and especially storage. However, losses in the viability of probiotic bacteria in ice cream unavoidably occur during product formulation, processing, storage and melting. During these stages, probiotic cells are subjected to different stresses related to pH, acidity, redox potential, freezing, oxygen (especially in overrun processing), sugar concentration and osmotic effects, hydrogen peroxide, antagonistic impact of co-cultures (in fermented ice creams), and mechanical shearing. It seems that the rate of loss of probiotic cells is greater during the freezing process than during storage. Practicing methods such as selection and application of oxygen-resistant probiotic strains, elimination of molecular oxygen (using oxygen-scavenging components, packaging material that is impermeable to oxygen as well as thicker packaging materials and active packaging systems), applying severe heat treatment, using microencapsulation techniques, and adjusting the product formulation (e.g., fortification of milk with nutrients and prebiotics) can increase the viability of probiotics in the final product. Supplementation of ice cream with probiotic bacteria has been found to have little effect on its flavor, texture or other sensory characteristics. There are also many ways to improve the sensory attributes of the product to compensate for any changes that do occur. This article reviews the viability of probiotic bacteria in ice cream and the main methods used to improve their viability and the sensory characteristics of probiotic ice cream.

Keyword Probiotic ice cream · Sensory · Viability

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Introduction

Worldwide, the demand for functional foods is growing rapidly due to consumers' increased awareness about the impact of food on health (Halsted 2003; Stoon 2002). Functional foods (also known as 'pharma foods' or 'nutraceuticals') are foods containing ingredient(s) that can prevent and/or treat diseases (Scheinbach 1998). Today, there has been a strong increase in the consumption of probiotic bacteria using food products, including probiotic dairy products such as fermented milks, ice cream, various

types of cheese, baby-food milk powder, frozen dairy desserts, whey-based beverages, sour cream, buttermilk, normal and flavored liquid milk, and concentrated milk. Among probiotic dairy products, probiotic ice cream is gaining popularity: ice cream can be stored for a long time without changes in its attributes, and it is a very popular product worldwide. Ice cream is an ideal matrix for delivery of probiotic organisms to the human body compared to fermented dairy products (Akin et al. 2007; Haynes and Playne 2002; Hekmat and McMahon 1992; Kailasapathy and Sultana 2003).

'Probiotics' can be defined as live microorganisms (bacteria and/or yeasts) that can bring health benefits to humans' or animals' bodies, usually the maintenance and/or improvement of the microbial balance of the intestine environment (Fuller, 1989; Gardiner et al. 2002; Holzapfel and Schillinger 2001; Mortazavian and Sohrabvandi 2006; Shah 2007). *Lactobacillus* and *Bifidobacterium* are the most common species of bacteria used as probiotics for the production of fermented milks and other dairy products (McFarland and Elmer 2006). Several generic health advantages are attributed to probiotics, including anti-mutagenic and anti-carcinogenic effects, immune system stimulation (immune modulation), anti-infection properties, serum cholesterol reduction, alleviation of lactose intolerance symptoms, and nutritional enhancements (Ishibashi and Shimamura 1993; Korbekandi et al. 2011; Mortazavian et al. 2010; Saarela et al. 2000; Shah 2007; Vinderola et al. 2000a).

The viability of probiotic bacteria, the number of viable and active cells per gram or milliliter of probiotic food products at the moment of consumption is the most critical value for these products, as it determines their efficacy (Mortazavian and Sohrabvandi 2006; Tamime et al. 2005). Therefore, in order to maintain consumer confidence in probiotic products, it is important to ensure a high survival rate of the bacteria both during production and over the product's shelf life (Saxelin et al. 1999). The minimum necessary concentration of probiotic bacteria to cause a beneficial result has been generally accepted as 10^6 viable cells/g-ml product at the moment the product is consumed (Blanchette et al. 1996; Gomes and Malcata 1999; Hekmat and McMahon 1992; Kurman and Rasic 1991; Rybka and Kailasapathy 1995). Also, many authors suggest that ingestion of 10^8-10^9 viable cells per day is needed to develop beneficial effects for humans (Oliveira et al. 2001; Saxelin 1997; Vinderola et al. 2000b). Apart from the viability of probiotics in products until the time of consumption, their survival after exposure to gastrointestinal tract conditions is also crucial. This article reviews the survival of probiotic bacteria in different types of ice cream and the main methods used to improve their viability and the sensory attributes of probiotic ice cream.

Viability of probiotic bacteria in ice cream from formulation to time of consumption

Maintaining the viability of probiotic cultures in food until the end of shelf life is an important criterion for providing effective probiotic food products. The majority of studies regarding viability of probiotic bacteria in ice cream have been associated with their survival rate in products obtained by different techniques such as culturing ice cream mix (Akin 2005; Davidson et al. 2000; Fávaro-Trindade et al. 2006; Hekmat and McMahon 1992), using non-fermented ice cream mix (Alamprese et al. 2002; Haynes and Playne 2002), or adding fermented milk to regular ice cream mix (Christiansen et al. 1996; Hagen and Narvhus 1999). Figure 1 shows the process flow diagram for production of fermented and non-fermented ice creams. Table 1 lists selected publications on probiotic bacteria used in ice cream. Various investigations have shown that probiotic cultures can better maintain their stability at appropriate level in frozen food products compared to probiotic fermented milks (Alamprese et al. 2002; Aryana and Summers 2006; Hekmat and McMahon 1992; Kailasapathy and Sultana 2003). The loss of viability of probiotic organisms in ice cream occurs either during the production stage or during frozen storage. Also, melting of the product at the moment of consumption could be detrimental to probiotic cells (Jay et al. 2005). At each stage, probiotic cells are subjected to different stress factors, including detrimental chemical compounds in the product formula (e.g., pH, titrable acidity and sugar content), freezing injuries, oxygen toxicity and mechanical stresses (shearing). Therefore, during frozen storage, temperature oscillations that lead to periodic partial melting and re-freezing should be avoided.

Table 2 summarizes the main factors that decrease the viability of probiotic bacteria in ice cream and their mechanisms of impact. The effects of the most significant factors affecting the viability of probiotic bacteria in ice cream from formulation to consumption are discussed in the following sections.

Effects of formulation

Sugars (e.g., lactose and particularly sucrose), which are used as key ingredients in dairy desserts and ice cream, have complex effects on the viability of probiotics in frozen products. While they might reduce probiotic survival because osmotic stress can affect cell viability (Jay et al. 2005), they might at the same time increase a probiotic's viability through acting as cryoprotectant (Champagne and Rastall 2009). The final effect might depend on the type and concentration of sugars, type of probiotic organism, freezing temperature and rate, as well

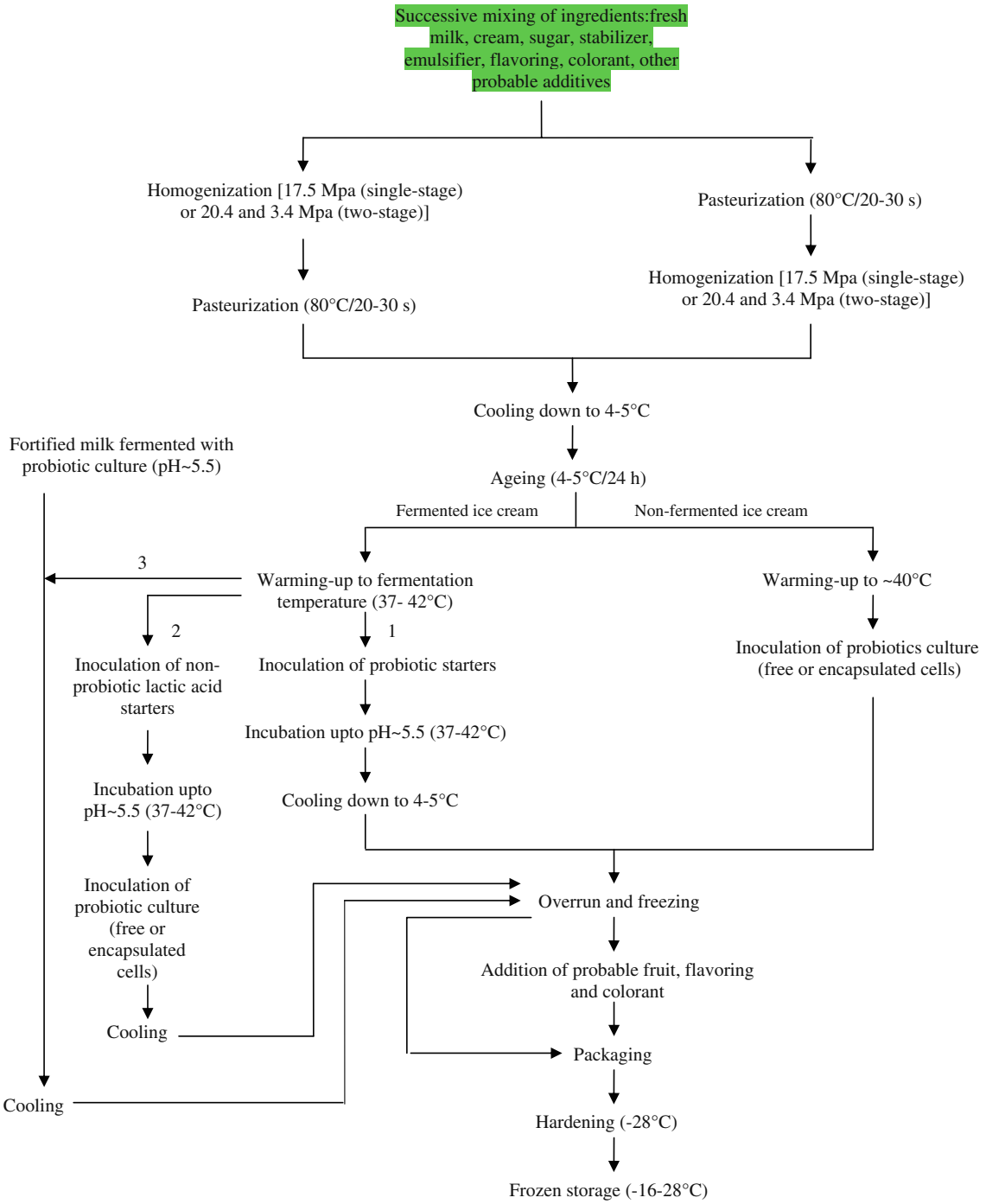

Fig. 1 Process flow diagram for manufacture of probiotic ice cream

as freezing technology and storage time. It has been proven that short-chain polysaccharides are more cryo-protective than long-chain polysaccharides (Champagne and Rastall 2009). Short-chain polysaccharides deemed 'prebiotic compounds' (e.g., fructo-oligosaccharides, see below) have the most beneficial effects on probiotic stability. However, the impact of these carbohydrates on product texture when they are used as cryoprotectant and/or prebiotics should not be overlooked.

The pH and titrable acidity of probiotic products can significantly affect cell survival of probiotic bacteria in fermented ice cream. The optimum pH for growth of *Lactobacillus acidophilus* is 5.5–6.0, but 6.0–7.0 for bifidobacteria. *L. acidophilus* is generally more tolerant of

Table 1 Selected publications on probiotic microorganisms used in ice cream. *CFU* Colony forming units

Formulation specifications	Probiotic bacteria	Storage conditions	Viability	Reference
Sucrose=13% (w/w); milk fat=4%; milk solid nonfat=12%; stabilizer/emulsifier=0.65%; inulin=4% ; final pH=5.5	*Lactobacillus acidophilus* La-5 *Bifidobacterium animalis* Bb-12	12 weeks at -18°C	>10^6CFU/g (in samples supplemented with inulin)	Akalin and Erişir (2008)
Sugar=18%; whole milk=45%; fat=15%; skim milk powder=7.4%; stabilizer=0.5%; final pH=5.8-6; inulin=2%	*Bacillus lactis* Bl-0l*L. acidophilus* La-14	21 weeks at -18°C	>10^6CFU/g (in the samples supplemented with inulin)	Akin et al. (2007)
Total sugar=15%; fat=10%; pasteurized skim milk=65%; skim milk powder=4.6%; non-fermented ice cream	*Lactobacillus johnsonii* La-1	32 weeks at -16°C and -28°C	>10^7CFU/g	Alamprese et al. (2002)
Total sugar=15%; Fft=10%; pasteurized skim milk=65%; skim milk powder=4.6%; non-fermented ice cream	*Lactobacillus rhamnosus* GG	48 weeks at -16°C and -28°C	>10^8CFU/g	Alamprese et al. (2005)
Sucrose=15%; milk fat=6%; nonfat milk solid=10%; aspartame=4%; stabilizer=0.3%; vanillin=0.3%; Final pH=5.6	*L. acidophilus* AB5-18 *L. acidophilus* AK4-14 *Lactobacillus agilis* AC18-88 *L. agilis* AA17-73 *L. rhamnosus* AB-20-100	24 weeks at -20°C	>10^8CFU/g	Başyğit, et al. (2006)
Sucrose=19%; pasteurized milk=65%; Fat=5%; emulsifier=0.3%;stabilizer=0.4%; final pH=5.5, 5 and 4.5	*Bifidobacterium longum* BL-04 *Bifidobacterium lactis* BL-01	15 weeks at -18°C	>10^6CFU/g (even in samples with pH 4.5)	Fávaro-Trindade et al. (2006)
Sucrose=10; glucose=4; Skim milk powder=5.5; whey protein powder=4.2; stabilizer=0.5 or 1%); inulin=6.2%; maltodextrin=3%; final pH=6.5	*L. acidophilus* La-5 *B. lactis* Bb-12 *Lactobacillus paracasei* CRL-431	52 weeks at -25°C	>10^6CFU/g	Haynes and Playne (2002)
Fat=12%; milk solid nonfat=11%; sugar=12.5%; corn syrup=4.5%; stabilizer=0.32%; final pH=5.5	*L. acidophilus* 10LF *Bifidobacterium bifidum* 10LF	17 weeks at -29°C	> 10^6CFU/g –10^7CFU/g	Hekmat and McMahon (1992)
Fresh milk=55%; cream=20%; sucrose=17%; resistant starch=1%; carrageenan and vanillin=0.1%; non-fermented ice cream	*L. casei* Lc-0 (encapsulated) *B. lactis* Bb-121(encapsulated)	42 weeks at -20°C	>10^6CFU/g >10^7CFU/g	(Homayouni et al. 2008a, 2008b)
Fat=8%; nonfat milk solid=11.2%; sucrose=14%; stabilizer=0.4%; final pH=6.5	*L. acidophilus* La-5 *B. animalis* Bb-12	8 weeks at -25°C	>10^6CFU/g –10^7CFU/g	Magriños et al. (2007)
Fat=8%; milk solid nonfat=12%; sucrose=16%; stabilizer/emulsifier=0.8; vanilla=0.3; final pH=5.6	*Lactobacillus gasseri* B-14168 *L .rhamnosus* B-445 *Lactobacillus reuteri* B-14171 *L. acidophilus* La-5 *B. bifidum* Bb-12	12 weeks at -26°C	>10^5CFU/g >10^6CFU/g >10^6CFU/g >10^5CFU/g >10^6CFU/g	Salem et al. 2005
Fat=6% or 8.5%; sucrose=9%; nonfat milk solid=11.59; stabilizer/emulsifier=0.4; skim milk powder=5%; final pH~6	*L. acidophilus* (DSMZ 20079) *B. bifidum* (DSMZ 200456)	90 days at -20°C	>10^6CFU/g	Turgut and Cakmakci (2009)

Table 2 Main factors decreasing viability of probiotic microorganisms in ice cream and their mechanisms of impact

State of damage	Stress factors	Resultant damage(s)	Methods of viability improvement	Selected sources
During the fermentation process (fermented ice cream)	Low pH values (especially less than ~5.5)	Mass transfer disruption through the cells, inactivation of some bacterial enzymes (cell starvation)	Selection of tolerant probiotic strains as well as suitable probiotic and/or non-probiotic starter cultures, addition of growth factors as well as growth promoters including prebiotics, finishing the milk fermentation at relatively higher pH values (~5.5)	Akalin and Erişir 2008; Alamprese et al. 2002; Champagne and Rastall 2009; Crittenden et al. 2001; Hagen and Narvhus 1999; Haynes and Playne 2002; Homayuni et al. 2008a; Medici et al. 2004; Stanton et al. 2003; Ziemer and Gibson 1998
	High titrable acidity	Bacteriocidic impact after entrance into bacterial cells		
	High redox potential (anaerobic bacteria)	Restriction and inactivation of cellular metabolic pathways		
	High concentration of sugars	Osmotic stress		
	Presence of food additives that are detrimental to probiotic cells	Damage to cell walls or other parts of the cells		
	Antagonistic interactions among starter cultures (fermented ice cream)	Loss of cell viability due to production of detrimental chemical compounds as well as nutritional competition		
During the freezing process	Mechanical damage caused by formation of ice crystals (including freezing rate) and by scraping of the cylinder wall	Bacteria cell walls rupture	Selection of probiotic strains tolerant to freezing, use of microencapsulated probiotic cells, use of cryoprotective carbohydrates, applying adequate technology of freezing regarding ice cream freezers	Akalin and Erişir 2008; Akin et al. 2007; Champagne and Rastall 2009; Gill 2006; Jay et al. 2005; Homayouni et al. 2008a, b; Krasaekoopt et al. 2003; Lankaputhra and Shah 1996
	Temperature-related stresses	Temperature decrease shock as well as the effects of frozen temperatures		
	Chemical and biochemical stresses	Condensation of detrimental solutes, dehydration of cells		
During the overrun process	Oxygen toxicity for anaerobic bacteria	Sensitivity of bacterial cells to metabolically produced hydrogen peroxide	Selection of oxygen-tolerant strains, use of microencapsulated probiotic cells, use of oxygen scavengers and redox potential reducing agents, use of packaging materials impermeable to oxygen	Akalin and Erişir 2008; Davies and Obafemi 1985; Haynes and Playne 2002; Laroia and Martin 1991; Miller et al. 2003; Ravula and Shah 1998; Vasiljevic and Shah 2008;
During frozen storage period	Time	Cells damaged during freezing die gradually during storage	Selecting tolerant probiotic strains, utilizing microencapsulated probiotic cells, avoiding temperature oscillations during storage of the product, using oxygen scavengers and redox potential reducing agents, applying packaging materials impermeable to oxygen	Akalin and Erişir 2008; Dave and Shah 1998; Davies and Obafemi 1985; Haynes and Playne 2002; Laroia and Martin 1991; Miller et al. 2003; Ravula and Shah 1998; Shah 2000; Vasiljevic and Shah 2008;
	Oxygen toxicity for anaerobic bacteria	Sensitivity of bacterial cells to metabolically produced hydrogen peroxide		
During melting/thawing of the product	Chemical stresses	Osmotic stress, condensation of detrimental solutes		Champagne and Rastall 2009; Jay et al. 2005

acidic conditions than bifidobacteria, the growth of which is significantly retarded below pH 5.5. The tolerance of *Bifidobacterium* spp. to acidic conditions is strain specific (Korbekandi et al. 2011). However, the pH of fermented ice cream is not as low as that of fermented milks or fermented frozen dairy desserts (about 5.5 in ice cream, compared with 4.5 or less in fermented milks and fermented frozen dairy desserts), resulting in considerably higher viability of probiotic bacteria in ice cream (Kailasapathy and Sultana 2003; Laroia and Martin 1991). For instance, in a study by Fávaro-Trindade et al. (2006), ice cream samples containing acerola pulp were formulated with the use of different starter cultures (*Bifidobacterium longum*, *Bifidobacterium lactis*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). Fermentation by the culture combinations was interrupted when pH values of 5.0 to 5.5 were reached. The addition of acerola pulp caused a decrease in the pH value to 4.5 and 5.0, respectively. The viability of the cultures remained above the recommended minimal limit of 10^6CFU/g for 15 weeks at a pH value of 4.5.

Some researchers have indicated that the fat content in ice cream does not significantly affect probiotic stability, particularly during storage. For example, Fávaro-Trindade et al. (2007) indicated that a higher concentration of fat did not provide greater protection to the probiotic bacteria. Similar results were obtained by Hayenes and Playne (2002), who found that full-fat ice cream mixes with 5–10% cream offered no extra protection for probiotic bacteria during storage when compared to those prepared with 3.8% fat. However, these observations disagree with those of Turgut and Cakmakci (2009), who investigated the effect of different cream levels (5% and 10%) and different strains of probiotic bacteria (*L. acidophilus*, *Bifidobacterium bifidum* and both) on the viability of probiotics in ice cream production during storage (1, 15, 30, 45, 60, 75 and 90 days). The results showed that counts of *B. bifidum* were higher with 10% cream throughout the storage time. *L. acidophilus* had high survival rates with 5% cream added on the 1st day, and the value decreased gradually throughout the storage (above 10^6CFU/g during 90 days). It seems that the impact of fat content on viability of probiotics should depend on probiotic strains as well as on other process and formulation conditions.

Effects of the freezing process

During the freezing process, the cells of probiotics can be lethally injured by damage to their cell walls or membranes caused by the mechanical stresses of ice crystals forming in the external medium or inside the cells, by cold injuries and temperature decrease shock to the cells, by condensation of solutes (those that are detrimental for probiotic cells) in the extracellular/intracellular medium, or by dehydration of the cells. All these factors cause reduction in the cells' vital metabolic activities (Akin et al. 2007; Davies and Obafemi 1985; Gill 2006; Jay et al. 2005). The size of the ice crystals increases as the freezing rate decreases; larger intracellular ice crystals cause greater damage to the cells (Gill 2006; Jay et al. 2005). Therefore, rapid freezing of the ice cream mix after inoculating with the probiotic bacteria contributes to the maintenance of the populations of these microorganisms in the product. One study reported that the rate of probiotic cell death was greater during the freezing process than during storage, and major freeze damage occurred when probiotics were in the ice cream freezer. This damage was caused mainly by the formation of ice crystals as well as by the scraping of the cylinder wall by the freezer blades (Homayouni et al. 2008a).

In general, probiotics' resistance to freezing damage differs among probiotic strains. Microorganisms that exhibit better ability to survive in freezing conditions are those that can dehydrate with no breakage of their cytoplasm membranes. Such cells can reduce the number and growth of intracellular ice crystals, and therefore reduce heat transfer through their cells; both factors minimize the damage to the microbial cells (Jay et al. 2005).

Magariños et al. (2007) investigated the viability of probiotic bacteria (*Lactobacillus acidophilus* La-5 and *Bifidobaterium animalis* ssp. *lactis* Bb-12) in ice cream (with 8% fat content). The freezing and inclusion of 108% overrun and the subsequent hardening of the ice cream permitted a survival rate of 91.3% (about 0.6 log cycles) of the *L. acidophilus*. Similar results were obtained by Hekmat and McMahon (1992), who indicated that the ice cream freezing process (with a mixture containing 12% fat) caused a reduction of about 1 log cycle in the total viable count of *L. acidophilus*. For *B. lactis*, a survival rate of 90.1% was determined after freezing and the incorporation of a 106% overrun. This finding agrees with that reported by Modler et al. (1990), who indicated that 10% of the initial count of bifidobacteria was lost in ice cream after production.

Effects of the overrun process

Apart from freezing injuries to probiotic cells, the incorporation of oxygen into the mix (overrun process) may result in an additional decrease in viable cell counts of probiotic bacteria, because oxygen content and redox potential (which is directly proportional to oxygen amount) are important factors in the viability of bifidobacteria during processing and storage. Most probiotic strains belong to the *Lactobacillus* and *Bifidobacterium* genera and they are often gut-derived organisms with microaerophilic or anaerobic metabolism; therefore, molecular oxygen as well as high values of redox potential

are critical factors for theses bacteria, particularly for bifidobacteria (Champagne and Gardner 2005; Cruz et al. 2007; Kawasaki et al. 2006; Mortazavian and Sohrabvandi 2006; Talwalkar and Kailasapathy 2004; Vasiljevic and Shah 2008). Unlike aerobic microorganisms, which completely reduce oxygen to water, for bifidobacteria the absorption system of this substance is minimal or even absent. The absence of an electron transport chain results in the incomplete reduction of oxygen to hydrogen peroxide. In addition, these bacteria do not produce catalase, an enzyme essential to the breakdown of hydrogen peroxide; this consequently leads to the accumulation of derived toxic metabolites such as superoxide anions, hydroxide radicals and hydrogen peroxides in the cell, causing its death (Champagne and Gardner 2005; Cruz et al. 2007). However, according to several different studies, inclusion of 106–108% overrun did not decrease the viability of probiotics (*L. acidophilus* and *B. lactis*) more than 10% after freezing and hardening (Hekmat and McMahon 1992; Magariños et al. 2007; Modler et al. 1990).

Effects of storage duration

The behavior of probiotic populations generally shows good survivability in ice cream up to the end of shelf life. During storage, variations in the survival time of probiotic bacteria depend on the strain, the production technology, storage temperature, storage time and product formulation (Christiansen et al. 1996; Hagen and Narvhus 1999; Hekmat and McMahon 1992). Research has revealed that, although the counts of probiotic cells decrease significantly throughout storage, the freezing and mixing involved in converting the mix into ice cream have a greater effect on viability loss (Akalin and Erişir 2008; Alamprese et al. 2002; Hagen and Narvhus 1999; Haynes and Playne 2002). Hekmat and McMahon (1992) determined the survival of *L. acidophilus* and *B. bifidum* in ice cream intended as a probiotic food product. The probiotic ice cream was prepared after fermentation of the ice cream base mix by both cultures, and was then submitted to frozen storage. Bacterial counts made immediately after freezing of the fermented mix were 1.5×10^8 for *L. acidophilus* and 2.5×10^8CFU/mL for *B. bifidum*. After 17 weeks of storage at −29°C, these populations decreased to 4×10^6 and to 1×10^7CFU/mL, respectively. Salem et al. (2005) investigated probiotic ice cream manufactured by mixing fortified milk fermented with probiotic strains (ice cream mix), followed by freezing. The viable counts decreased 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 (−26°C). Although there was a decrease in the number of viable cells, the ice cream could be considered as a probiotic food during the storage period, since the number of viable probiotic cells remained above the recommended minimum limit of 1×10^6CFU/g. Fávaro-Trindade et al. (2007) investigated fermented yellow mombin (*Sponadias mombin* L) ice cream produced by different starter cultures (*L. acidophilus* 74-2, *L. acidophilus* LAC4 and yogurt starter cultures), with different pH values (4.5 and 5) and different concentrations of added cream (5 and 10%). The mixes were frozen and stored for 105 days at −18°C. The initial culture counts ranged from 10^7 and 10^9CFU/g and showed a tendency to decrease throughout the storage period, with reductions ranging from 0.6 to 3 logarithmic cycles. However, all the samples maintained counts higher than 10^6CFU/g. These results were similar to those obtained by Christiansen et al. (1996), Hagen and Narvhus (1999), Haynes and Playn (2002), Kailasapathy and Sultana (2003), Akin (2005), and Fávaro-Trindade et al. (2006). Most of these studies have shown that probiotic bacteria can survive in ice cream for up to 6 months in frozen conditions (−18 to −28°C) and remain above the recommended minimum limit of 10^6CFU/g.

Effects of ice cream melting during consumption

Probiotic cells are subjected to some chemical stresses during melting (freeze–thaw) of the frozen; these stresses can kill them. During melting, the cells are exposed to osmotic effects (Jay et al. 2005); moreover, the freezing concentration associated with melting results in high concentrations of detrimental factors such as hydrogen ions, organic acids, oxygen and other components poisonous to probiotic cells. These factors have a great effect on probiotic viability loss. In particular, pH has been found to exhibit a crucial role in this regard. Therefore, the lower the pH in ice cream mix, the higher the viability loss of probiotic cells during melting of the product. It has been reported that, in frozen yogurt with pH of about 4.2 to 4.5, the viability of probiotics after thawing is markedly lower than in ice cream (Champagne and Rastall 2009).

Main methods to improve the viability of probiotic bacteria in ice cream

A number of methods to improve the viability of probiotic bacteria in ice cream are discussed below:

Selection of resistant bacterial strains

Suitable probiotic strains are those that can maintain their survival and stability during commercial production of products as well as during the storage period (Godward et al. 2000; Talwalker and Kailasapathy 2004). Furthermore, a

high viable survival rate during delivery through the gastrointestinal tract is necessary to allow enough live cells to arrive at the human intestine. Therefore, selection of resistant probiotic strains that can tolerate production, storage and gastrointestinal tract conditions is of prime importance. Research has indicated that the survival of bacteria in unfavorable conditions such as freezing, oxygen toxicity and storage at lower temperatures is species- and strain-specific (Haynes and Playne 2002; Kailasapathy and Sultana 2003; Ravula and Shah 1998). Generally, lactobacilli strains have proven to be highly resistant to detrimental conditions in comparison with bifidobacteria strains in frozen products (Homayouni et al. 2008b; Tamime et al. 2005). Properly selected strains, such as *Lactobacillus johnsonii* La-1, survive satisfactorily in the relatively high sugar content of ice cream, and can withstand the sub-lethal injuries caused by freezing during the production of this product. For instance, counts of $>10^7$CFU/g were maintained for at least 10 weeks (El-Shazly et al. 2004; Hamed et al. 2004; Rao and Prakash 2004) or 8 months (Alamprese et al. 2002; 2005; Haynes and Playne 2002) of storage. Some strains of probiotic bacteria did not withstand the freezing process (Hagen and Narvhus 1999; Haynes and Playne 2002), but some others such as *B. longum* and *B. infantis* were able to survive these processes and storage for up to 11 (Davidson et al. 2000) or 52 (Haynes and Playne 2002) weeks. Acid and pH resistance in probiotic bacteria is strain-dependent, and bifidobacteria strains are more sensitive than *Lactobacillus* strains. *L. acidophilus* is reported to have a high cytoplasmic buffering capacity (pH 3.72–7.74), which allows it to resist changes in cytoplasmic pH and to gain stability under acidic conditions (Godward et al. 2000; Tamim et al. 2005). The influence of a particular enzyme, H^+-ATPase, has been identified in this regard (Takahashi et al. 2007).

Adjusting product formulation (modifying media composition)

It has been well-proven that supplementation of milk with different growth factors (used directly by probiotics as nutrients) and/or growth promoters (used to enhance growth and/or activity of probiotic cells but not used directly as nutrients) can significantly increase the stability of probiotic bacteria in food products. This method is especially under attention in fermented dairy products. Casein, whey protein hydrolysates, L-cysteine, yeast extract, glucose, some vitamins and probiotic compounds are among the most important growth factors/growth promoters (Mortazavian and Sohrabvandi 2006; Tamim et al. 2005). Milk supplemented with peptides and amino acids such as L-cysteine improve the survival of bifidobacteria (McFarland and Elmer 2006; Rao and Prakash 2004). Dave and Shah (1998) studied the effect of L-cysteine, whey protein concentrate, acid casein hydrolysate and tryptone on the viability of *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *L. acidophilus* and bifidobacteria in frozen dairy desserts. They reported that addition of these ingredients improved the viability of *L. acidophilus* and bifidobacteria by providing growth factors as these probiotic bacteria lack proteolytic activity (Dave and Shah 1998). Protein derivatives promote probiotic survival for several reasons: their nutritional value for the cells, their ability to reduce the redox potential of the medium, and their ability to increase the medium's buffering capacity (which results in a smaller decrease in pH) (Dave and Shah 1998; Mortazavian et al. 2011).

Prebiotics are non-digestible (or very low digestible) food ingredients (mostly oligosaccharides) that are selectively metabolized by probiotics and enhance their growth and/or activity. These compounds (such as fructooligosaccharides and galactooligosaccharides) can be added to probiotic products as important growth factors (Mortazavian and Sohrabvandi 2006), and can significantly improve the retention of probiotic viability (especially for bifidobacteria) in food products as well as in the gastrointestinal tract (Gibson and Roberfroid 1995; Gibson et al. 2004; Loo et al. 1999; Mizota 1996; Rycroft 2001). The addition of growth-promoting factors and prebiotics, such as inulin, has been shown to significantly improve the viability of probiotic organisms (Crittenden et al. 2001). Akin et al. (2007) investigated ice cream that contained probiotic bacteria (*Lactobacillus acidophilus* and *B. lactis*) and produced by mixing fortified milk fermented by probiotic strains with ice cream mixes with different sugar concentrations [15, 18 and 21% (w/w)]. Cultures were grown (37°C, 12 h) in UHT skim milk with or without the addition of inulin (1 and 2%). The results suggested that the addition of inulin stimulated the growth of *L. acidophilus* and *B. lactis*, which resulted in their improved viability. Palframan et al. (2003) reported similar results. In another study, addition of inulin or oligofructose improved probiotic organisms' ability to withstand freezing even in acidic ice cream (pH 5.5) (Champagne and Rastall 2009).

The concentration of sugars as well as the final pH of the product require particular attention due to their significant effects on the viability of probiotics in ice cream. Although some sugars (e.g., sucrose and lactose) show a cryoprotective effect on probiotics in frozen products, a relatively high sugar content can adversely affect the viability of probiotics due to excessive increases in osmotic pressure (see above). The inhibitory impact of sugars on probiotic growth is more pronounced in probiotic fermented ice cream, because probiotics are added as a starter culture to multiply in the medium (Fig. 1). The pH of ice cream mix in fermented ice cream must be closely controlled during the fermentation

process, because a pH below about 5.5 leads to significant loss of probiotic viability.

Elimination of molecular oxygen

The freezing process for frozen dairy desserts incorporates air into the final product. Proximate overrun could be 100%. Oxygen comprises 21% of air. The use of oxygen scavengers such as L-ascorbic acid and potent redox potential reducing agents such as L-cysteine in the production of dairy products, along with the use of packaging material impermeable to oxygen can reduce the amount of oxygen in dairy products (Miller et al. 2003; Mortazavian et al. 2011; Shah 2000). Media used for the enumeration of bifidobacteria often contain L-cysteine (0.5–0.1 g/100 mL) in order to improve bacterial recovery, as L-cysteine provides amino nitrogen, a growth factor for probiotic cells (in particular, an essential amino acid for bifidobacteria), and efficiently reduces a medium's redox potential. The use of 50 mg/kg L-cysteine in probiotic dairy products is recommended (Dave and Shah 1998; Ishibashi and Shimamura 1993; Laroia and Martin 1991; Mortazavian et al. 2011; Ravula and Shah 1998; Tamime et al. 2005). Applying severe heat treatment and deaeration to milk used for fermented frozen dairy desserts, controlling production to achieve minimum dissolved oxygen in milk, and co-culturing *S. thermophilus* with bifidobacteria (as an efficient oxygen consumer) are some methods to deplete molecular oxygen as well as to reduce redox potential in probiotic products (Tamime et al. 2005).

Packaging plays a fundamental role in the viability of probiotic bacteria in ice cream. The level of oxygen within the package during the storage period of the product should be as low as possible in order to avoid toxicity and the death of probiotic bacteria. Using packaging materials impermeable to oxygen, increasing the thickness of packaging materials and applying active/intelligent packaging systems using oxygen scavengers (in sachets suspended under container lids or incorporated into packaging polymers) or using a combination of such techniques, are methods used to efficiently decrease molecular oxygen in probiotic products (Cruz et al. 2007; Miller et al. 2003; Talwalkar et al. 2004).

Microencapsulation

Microencapsulation—one of the newest and most efficient methods for increasing the viability of probiotics—has recently come under particular consideration and investigation. From a microbiological point of view, microencapsulation can be defined as the process of entrapping or enclosing microorganisms by segregating them from their environment with a coating of hydrocolloid(s) in a way that results in appropriate cell release in the intestinal medium (Krasaekoopt et al. 2003; Mortazavian et al. 2007, 2008; Picot and Lacroix 2003; Sultana et al. 2000). Microencapsulation of probiotic cells has been shown to preserve them from detrimental environmental factors such as low pH and high acidity (Wenrong and Griffiths 2000), bile salts (Lee and Heo 2000), cold shocks induced by process conditions such as deep freezing and freeze drying (Shah and Rarvula 2000), molecular oxygen in the case of obligatory anaerobic bacteria (Sunohara et al. 1995), heat shocks caused by process conditions such as spray drying, bacteriophages (Steenson et al. 1987) and chemical antimicrobial agents (Sultana et al. 2000). In addition, other advantages such as improvement and stabilization of sensory properties (Gomes and Malcata 1999) and immobilization of the cells, which facilitates their homogeneous distribution throughout the product (Krasaekoopt et al. 2003), can also be achieved by this process.

According to several findings, encapsulation is a useful alternative to increase the survival rate of probiotic bacteria in ice cream and fermented frozen dairy desserts. When incorporated into fermented frozen dairy desserts, encapsulated probiotic organisms showed an improved viability of $>10^5$CFU/g in the product, compared to counts of $<10^3$CFU/g when non-encapsulated organisms were used (Mortazavian et al. 2011; Shah and Ravula 2004). Evaluation of the encapsulation of different microorganisms (*L. acidophilus* 2401, 2404, 2409, and 2415, *L. infantis* 1912; *B. animalis* ssp. *lactis* 1941; 920 and Bb-12; and *B. longum* 5581) have shown that free cells and freshly encapsulated cells without freeze-drying exhibit the best survival rates (Godward and Kailasapathy 2000; Talwalker and Kailasapathy 2003; Kailasapathy and Sultana 2003). Homayouni et al. (2008a) investigated the effect of microencapsulation on the survival of two probiotic strains added to a synbiotic ice cream. Two types of synbiotic ice cream containing 1% resistant starch with free and encapsulated *L. casei* Lc-01 and *B. lactis* Bb-12 were manufactured. Their survival was monitored during product storage for 180 days at −20°C. The probiotics' viability in the free and encapsulated states was 5.1×10^9 and 4.1×10^9CFU/g, respectively, at day 1, and 4.2×10^6 and 1.1×10^7CFU/g, respectively, after 180 days of storage at −20°C. The microencapsulation of probiotic bacteria in calcium alginate beads increased their survival rate about 30% during the same period of storage at the same temperature. In addition, it was demonstrated that encapsulated cells required more time to decrease one log cycle in viable counts. Microencapsulation of probiotic bacteria in beads with a diameter of about 20 μm can increase the viability of probiotics (Homayouni et al. 2008a). Sheu and Marshall (1993) reported that a mean diameter of 30 μm for calcium alginate beads was

desirable for use in frozen dairy desserts. Larger beads might cause coarseness of texture, whereas smaller beads did not sufficiently protect the bacteria (Khosrokhavar and Mortazavian 2010c; Mortazavian et al. 2007).

Sensory characteristics of probiotic ice cream

Tests to determine whether consumers will like a product are a necessary step in formulating a successful food product (Yackinous et al. 1999). Consumer testing, valuable in determining a product's sensory characteristics, usually incorporates discrimination and descriptive tests (Stone and Sidel 2004). These tests indicate which sensory characteristics and levels of these characteristics a product should exhibit in order to be successful in the market place. Some studies (Cruz et al. 2010; Tuorila and Cardello 2002) have shown that flavor is the first indicator when consumers choose a food, followed by health benefits. These studies also indicated that consumers are not interested in consuming a functional food if the added ingredients confer disagreeable flavors on the product, even if this results in health benefits. In general, incorporating prebiotic ingredients into ice cream has a greater influence on flavor and texture, whereas adding probiotics affects primarily flavor (Cruz et al. 2010). The metabolism of the probiotic cultures can result in the production of components that may contribute negatively to the taste and aroma of the product, e.g., acetic acid is produced by *Bifidobacterium* spp. during fermentation and storage. The *Bifidobacterium* fermentation pathway results in 3 mol acetic acid and 2 mol lactic acid per 2 mol glucose in an ideal synthetic medium, generating a theoretical molar ratio of acetic acid to lactic acid of 3:2 (Tamime et al. 2005). The acetic acid causes a "vinegary taint" if its concentration is high enough. Davidson et al. (2000) reported that, in general, ice creams fermented with probiotic cultures presented weaker aroma and taste (poor sensory performance) compared to those fermented with traditional yogurt starters. Thus, the production of probiotic ice creams with high sensory acceptance is a difficult task (Aryana and Summers 2006; Fávaro-Trindade et al. 2006; Hekmat and McMahon 1992). However, it is possible to develop probiotic ice cream with good sensorial quality (Christiansen et al. 1996; Vardar and Öksüz 2007).

Salem et al. (2005) manufactured probiotic ice cream by mixing fortified milk fermented with probiotic strains (*Lactobacillus reuteri*, *B. bifidum*, *L. acidophilus* and *Lactobacillus rhamnosus*) with an ice cream mix, followed by freezing. The probiotic ice cream was evaluated for chemical properties, culture survival and sensory characteristics during 12 weeks of frozen storage at −26°C. Ice cream prepared with *L. reuteri* and *B. bifidum* showed the highest sensory scores for flavor, body, texture, color and melting quality among all treatments. On the other hand, probiotic ice cream containing *L. rhamnosus* was the least-preferred sample and obtained the lowest score, especially for flavor. The probiotic taste was not found to be particularly noticeable in any bio-ice-cream samples. One reason for this could be the high pH value of the resultant ice cream. All ice cream samples supplemented with the probiotic strains were acceptable and gave a good total impression with no marked off-flavor. These findings were in line with those of Hagen and Narvhus (1999). Vardar and Öksüz (2007) reported that artisan strawberry ice cream supplemented with *L. acidophilus* showed good sensory acceptance. The incubation of the mix at pH 5.6 led to better results for flavor and taste. The authors suggested that adding highly acidic fruit to ice cream might be useful in masking the sour taste resulting from the metabolism of probiotic cultures. However, these results should not be generalized, as opposite findings were reported by Fávaro-Trindade et al. (2006) for probiotic ice cream with acerola fruit.

In ice cream manufacture, overrun affects the structure of the final product, since the presence of air gives the ice cream an agreeable light texture as well as melting and hardness characteristics that affect its sensory properties (Sofjan and Hartel 2004). Salem et al. (2005) reported that overrun percent was affected by the addition of probiotic cultures. While the best and highest overrun was obtained for the mix containing *L. acidophilus* and *B. bifidum*, the lowest overrun was observed in that containing *L. reuteri*. Differences in overrun of bio-ice-cream were attributed to the different degrees of acidity of mixtures with different probiotic cultures; these differences affected the freezing point and/or the nature of proteins. However, Akalin and Erişir (2008) reported that the addition of *L. acidophilus* La-5 and *B. animalis* Bb-12 (fermentation of the mix) did not significantly affect overrun values. Alamprese et al. (2005) indicated that when *L. rhamnosus* GG was added to ice cream mixes at a quantity of 1×10^8CFU/g, it did not change the overrun, firmness or melting behavior of the finished product. Regardless of formulation, no count decay of *L. rhamnosus* GG cells was observed in ice cream stored for up to 1 year.

Many studies have focused on applying prebiotic compounds (see above) in the manufacture of bio-ice-cream (synbiotic ice cream) and the impacts of these compounds on probiotic stability, as well as on the product's sensory characteristics. Inulin and oligofructose, the best-known prebiotics (which also serve as fat replacers), possess several functional and nutritional properties that may be used to formulate innovative healthy foods. Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates. Inulin has a degree of

polymerization (DP) of between 2 and 60. Oligofructose, a sub-group of inulin, consists of polymers within a DP≤10. Both inulin and oligofructose are used widely in functional foods throughout the world (Sangeetha et al. 2005). Their structure is similar to corn sweeteners, the principal carbohydrates used in ice cream manufacture. Classified as fat replacers, inulin and oligofructose influence products' texture and mouthfeel. They are resistant to hydrolysis in both the stomach and the small intestine, and are classified as dietary-fiber ingredients (Spiegel et al. 1994; Niness 1999). Inulin exhibits no adverse effect on the sensory properties of probiotic ice cream at concentrations of about 1 or 2% (Akin et al. 2007; El-Nagar et al. 2002).The addition of oligofructose or inulin significantly increases the overrun in probiotic ice cream, indicating its responsibility for increased air incorporation (Akalin and Erişir 2008; Akin et al. 2007). Studies indicate that increasing the amount of inulin in ice cream mixes increase complete melting times. Inulin may act as a stabilizer due to its capacity for binding water; as a result, water molecules become immobilized and unable to move freely among other molecules in the mix (Akalin and Erişir 2008). The ice cream melting process can be described in relation to the freedom of movement of molecules. Inulin (which can reduce the free movement of water molecules) appears to retard product melting (El-Nagar et al. 2002). Due to its longer chain length, inulin is less soluble than oligofructose and has the ability to form inulin microcrystals when sheared in water or milk. These crystals interact to form a creamy texture (Niness 1999). In addition, the ability of inulin to bind water molecules and form a particle gel network can improve product firmness (Franck 2002). The viscosity of ice cream mixes is increased significantly with the addition of oligofructose or inulin. The high apparent viscosity in probiotic ice cream mixes containing oligofructose or inulin can be explained by the interactions of the dietary fiber and liquid components of the probiotic ice cream mix. Research has shown that ice cream mixes containing carbohydrate-based fat replacers exhibit high viscosity because of these fat replacers' capability for imbibing water, which increases the viscosity of the system (Schmidt et al. 1993). Significantly higher apparent viscosity of the ice cream mix has been obtained by replacing 100% of the 42 DE corn syrup with inulin in a reduced-fat mix (Schaller-Povolny and Smith 2001). Higher apparent viscosity resulted from the higher molecular weight of inulin; this suggests that a potential interaction between the inulin and milk proteins could also be present in the system. Inulin, being highly hygroscopic, would bind water and form a gel-like network. In addition to other components (like corn syrup or an emulsifier-stabilizer mixture), inulin can modify the rheology of the ice cream mix. Similar results in relation to the effect of inulin on viscosity were reported by El-Nagar et al. (2002) and Akin (2005) for yogurt-ice cream and probiotic fermented ice cream, respectively.

Conclusion

The incorporation of probiotic bacteria into different types of ice cream is highly advantageous: in addition to making a food rich in health benefits, the ice cream itself contains dairy raw material, vitamins and minerals, and it is consumed by the general population. Thus, ice cream can serve as an excellent environment to deliver probiotics into the human intestine. Production and frozen storage of ice cream have relatively little effect on probiotic survival compared to fermented milk products, and studies have shown that bacterial cultures remain at levels sufficient to offer the suggested therapeutic effects. Nevertheless, loss of probiotics viability in ice cream unavoidably occurs during processing and storage due to exposure of the cells to different stress factors associated with formulation, freezing, overrun, storage and melting. Probiotic stability in ice cream can be enhanced by several methods such as selection of robust bacterial strains, elimination of molecular oxygen, using microencapsulation techniques and adjusting the product formulation. Attempts are being made to extend the shelf life of probiotic ice cream while retaining the product's therapeutic benefits. Supplementing ice cream with probiotic bacteria has been found to have little effect on its flavor, texture or compositional characteristics. Also, there are many ways to improve the sensory attributes of the product. Ice cream can be supplemented with prebiotics (preferably inulin and oligofructose) to improve probiotic stability as well as the sensory and physicochemical characteristics of synbiotic ice cream.

References

Akalin AS, Erişir D (2008) Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream. J Food Sci 73:184–188

Akin S (2005) Effects of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensory characteristics of probiotic fermented ice cream. Milchwisssenschaft 60:297–301

Akin MB, Akin MS, Kirmaci Z (2007) Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chem 104:93–99

Alamprese C, Foschino R, Rossi M, Pompei C, Savani L (2002) Survival of *Lactobacillus johnsonii* La1 and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations. Int Dairy J 12:201–208

Alamprese C, Foschino R, Rossi M, Pompei C, Corti S (2005) Effects of *Lactobacillus rhamnosus* CG addition in ice cream. Int J Dairy Technol 58:200–206

Aryana KJ, Summers M (2006) Probiotic fat-free, no sugar added ice cream. Milchwissenchaft 61:84–187

Başyğit G, Kuleaşan H, Karahan AG (2006) Viability of human-derived probiotic lactobacilli in ice cream with sucrose and aspartame. J Ind Microbiol Technol 33:96–800

Blanchette L, Roy Q, Belanger G, Gauthier S (1996) Production of cottage cheese using dressing fermented by bifidobacteria. J Dairy Sci 79:8–15s

Champagne CP, Gardner NJ (2005) Challenges in the addition of probiotic cultures to foods. Crit Rev Food Sci Nutr 45:61–84

Champagne CP, Rastall RA (2009) Some technological challenges in the addition of probiotic bacteria to foods. In: Charalampopoulos D, Rastall RA (eds) Prebiotics and probiotics science and technology. Springer, Berlin, pp 763–806

Christianesen PS, Edelten D, Kristiansen JR, Nielsen EW (1996) Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. Milchwissenschaft 51:502–504

Crittenden RG, Morris LF, Harvey ML, Tran LT, Mitchell HL, Playne MJ (2001) Selection of a Bifidubacterium strain to complement resistant starch in synbiotic yoghurt. J Appl Microbiol 90:268–278

Cruz AG, Faria JAF, Van Dender AGF (2007) Packaging system and probiotic dairy foods. Food Res Int 40:951–956

Cruz AG, Cadena RS, Walter EHM, Mortazavian AM, Granato D, Faria, JAF, Bolini HMA (2010) Sensory analysis: relevance for prebiotic, probiotic and symbiotic product development. Compr Rev Food Sci Saf 9:358–373

Dave RI, Shah NP (1998) Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J Dairy Sci 81:2804–2816

Davidson RH, Duncan SE, Hackney CR, Eigel WN, Boling JW (2000) Probiotic culture survival and implications in fermented frozen yoghurt characteristics. J Dairy Sci 83:666–673

Davies R, Obafemi A (1985) Response of micro-organisms to freeze-thaw stress. In: Robinson RK (ed) Microbiology of frozen foods. Elsevier, pp 83–107

El-Nagar G, Clowes G, Tudorica CM, Kuri V (2002) Rheological quality and stability of yog-ice cream with added inulin. Int J Dairy Technol 55:89–93

El-Shazly A, El-Tahra MAA, Abo-Sera MM (2004) Effect of different methods for the manufacture of frozen yogurt on its properties. Egyptian Conference for Dairy Science Technology, Cairo, 9–11 October 2004, Research Papers I, pp 183–194

Fávaro-Trindade CS, Bernardi S, Bodini RB, de Carvalho Balieiro JC, de Almeida E (2006) Sensory acceptability and stability of probiotic microorganisms and vitamin C in fermented acerola (*Malpigha emarginata* DC.) ice cream. J Food Sci 71:492–495

Fávaro-Trindade CS, Balieiro JCC, Dias PF, Sanino FA, Boschini C (2007) Effects of culture, pH and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow mombin (*Spondias mombin* L) ice creams. Food Sci Technol Int 13:285–291

Franck A (2002) Technological functionality of inulin and oligofructose. Br J Nutr 2:287–291

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Gardiner GE, Ross RP, Kelly PM, Stanton C (2002) Microbiology of therapeutic milks. In: Robinson RK (ed) Dairy microbiology handbook. Wiley, New York, pp 431–478

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Gibson GR, Rabiu B, Rycroft CE, Rastall RA (2004) Trans-galactooligosaccharides as prebiotics. In: Shortt C, Brien JO (eds) Handbook of functional dairy products. CRC, Boca Raton, pp 91–109

Gill CO (2006) Microbiology of frozen foods. In: Da-Wen Boca S (ed) Handbook of frozen food processing and packaging. CRC, Boca Raton, pp 85–100

Godward G, Kailasapathy K (2000) Viability and survival of free, encapsulated and co-encapsulated probiotic bacteria in ice cream. Milchwissenschaft 58:161–164

Godward G, Sultana K, Kailasapathy K, Peiris P, Arumugaswamy R, Reynolds N (2000) The importance of strain selection on the viability of probiotic bacteria in dairy foods. Milchwissenschaft 55:441–445

Gomes AMP, Malcata FX (1999) *Bifidobacterium* spp and *Lactobacillus acidophilus:* biological and theraputical revalant for use a probiotics. Trends Food Sci Technol 10:139–157

Hagen M, Narvhus JA (1999) Production of ice cream containing probiotic bacteria. Milchwissenschaft 54:265–268

Halsted CH (2003) Dietary supplements and functional foods: 2 sides of a coin? Am J Clin Nutr 77(Suppl):1001S–1007S

Hamed AI, Zedan MA, Salem OM, Moussa AM, Yousef ETA (2004) Impact of frozen yoghurt ingredients on its quality and survival of bifidobacteria. ii. Effect of milk solids not fat sources. In: Proceedings of the Egyption Conference for Dairy Science and Technology: Milk and Dairy Products for a Healthly Future, 9–11 October, pp 227–242

Haynes IN, Playne MJ (2002) Survival of probiotic cultures in low fat ice cream. Aust J Dairy Technol 57:10–14

Hekmat S, McMahon D (1992) Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. J Dairy Sci 75:1415–1422

Holzaspfel WH, Schillinger U (2001) Introduction to pre- and probiotics. Food Res Int 35:109–116

Homayouni A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH (2008a) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of symbiotic ice cream. Food Chem 111:50–55

Homayouni A, Ehsani MR, Azizi A, Razavi SH, Yarmand MS (2008b) Growth and survival of some probiotic strains in simulated ice cream conditions. J Appl Sci 8:379–382

Ishibashi N, Shimamura S (1993) Bifidobacteria: research and development in Japan. Food Technol 47:126–136

Jay JM, Loessner MJ, Golden DA (2005) Modern food microbiology. Springer, New York

Kailasapathy K, Sultana K (2003) Survival of β-D-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream. Aust J Dairy Technol 58:223–227

Kawasaki S, Mimura T, Satoh S, Takeda K, Nimura Y (2006) Response of the microaerophilic *Bifidobacterium* species, *B. boum* and *B. thermolum*, to oxygen. Appl Environ Microbiol 72:6854–6858

Khosrokhavar R, Mortazavian AM (2010) Effects of probiotic-containing microencapsules on viscosity, phase separation and sensory attributes of drink based on fermented milk. Milchwissenschaft 65:177–179

Korbekandi H, Mortazavian AM, Iravani S (2011) Technology and stability of probiotic in fermented milks. In: Shah N, Cruz AG, Faria JAF (eds) Probiotic and prebiotic foods: technology, stability and benefits to human health. Blackwell, Oxford (in press)

Krasaekoopt W, Bhandari B, Deeth H (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13:3–13

Kurman JA, Rasic JL (1991) The health potential of products containing bifidobacteria. In: Robinson RK (ed) Therapeutic properties of functional milks. Elsevier, London, pp 117–158

Lankaputhra WEV, Shah NP (1996) A simple method for selective enumeration of *Lactobacillus acidophilus* in yogurt supplemented with *L. acidophilus* and *Bifidobacterium* spp. Milchwissenschaft 51:446–451

Laroia S, Martin JH (1991) Effect of pH on survival of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in frozen fermented desserts. Cult Dairy Prod J 26:3–21

Lee KI, Heo TR (2000) Survival of *Bifidobacterium longum* immobilized in calcium alginate beads in simulated gastric juices and bile salt solution. Appl Environ Microbiol 66:869–973

Loo JV, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M (1999) Functional food properties of non-digestible oligosaccarides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Br J Nutr 81:121–132

Magariños H, Selaive S, Costa M, Flores M, Pizarro O (2007) Viability of probiotic microorganisms (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* ssp. *lactis* Bb-12) in ice cream. Int J Dairy Technol 60:128–134

McFarland LV, Elmer GW (2006) Properties of evidence-based probiotics for human health. In: Goktepe I, Juneja VK, Ahmedna M (eds) Probiotics in food safety and human health. Taylor and Francis, New York, pp 109–138

Medici M, Vinderola CG, Perdigon G (2004) Gut mucosal immunomodulation by probiotic fresh cheese. Int Dairy J 14:611–618

Miller CW, Nguyen MH, Rooney M, Kailasapthy K (2003) The control of dissolved oxygen content in probiotic yogurts by alternative packing materials. Packag Technol Sci 16:61–67

Mizota T (1996) Functional and nutritional foods containing bifidogenic factors. Bull Int Dairy Found 313:31–35

Modler H, McKellar R, Goff H, Mackie D (1990) Using ice cream as a mechanism to incorporate bifidobacteria and fructooligosaccharides into the human diet. Cult Dairy Prod J 25:4–9

Mortazavian AM, Sohrabvandi S (2006) Probiotics and food probiotic products; based on dairy probiotic products. Eta, Tehran

Mortazavian AM, Razavi SH, Ehsani MR, Sohrabvandi S (2007) Principles and methods of microencapsulation of probiotic microorganisms. Iran J Biotechnol 5:1–18

Mortazavian AM, Ehsani MR, Azizi A, Razavi SH, Mousavi SM, Sohrabvandi S, Reinheimer JA (2008) Viability of calcium-alginate-microencapsulated probiotic bacteria in Iranian yogurt drink (Doogh) during refrigerated storage and under simulated gastrointestinal conditions. Aust J Dairy Technol 63:24–29

Mortazavian AM, Khosrokhvar R, Rastegar H, Mortazaei GR (2010) Effects of dry matter standardization order on biochemical and microbiological characteristics of freshly made probiotic Doogh (Iranian fermented milk drink). Ital J Food Sci 22:98–102

Mortazavian AM, Mohammadi R, Cruz AG, Faria JAF (2011) Technology and stability of probiotics in dairy desserts. In: Shah NP, Cruz AG, Faria JAF (eds) Probiotic and prebioticfFoods: technology, stability and benefits to human health. Nova Science Publishing Ltd (in press)

Niness KR (1999) Inulin and oligofructose: what are they? J Nutr 129:1402–1406

Oliveira MN, Sodini I, Remeuf F, Corrieu G (2001) Effect of milk supplementation and culture composition on acidification, textural properties and microbiological stability of fermented milks containing probiotic bacteria. Int Dairy J 11:935–942

Palframan R, Gibson GR, Rastall RA (2003) Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. Lett Appl Microbiol 37:281–284

Picot A, Lacroix C (2003) Effect of micronization on viability and thermotolerance of probiotic freeze-dried cultures. Int Dairy J 13:455–462

Rao HGR, Prakash AS (2004) Development of probiotic *kulfi* (Indian ice cream). Indian Dairyman 56:57–64

Ravula RR, Shah NP (1998) Effect of acid casein hydrolyzates and cysteine on the viability of yogurt and probiotic bacteria in fermented frozen dairy desserts. Aust J Dairy Technol 53:174–179

Rybka S, Kailasapathy K (1995) The survival of culture bacteria in fresh and freeze-dried AB yoghurts. Aust J Dairy Technol 50:51–57

Rycroft CE, Jones MR, Gibson GR, Rastall RA (2001) A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Microbiol 91:878–887

Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T (2000) Probiotic bacteria: safety, functional and technological properties. J Biotechnol 84:197–215

Salem MMF, Fathi FA, Awad RA (2005) Production of probiotic ice cream. Pol J Food Sci Nutr 55:267–271

Sangeetha PT, Ramesh MN, Prapulla SG (2005) Recent trends in the microbial production, analysis and application of fructooligosaccharides. Trends Food Sci Technol 16:442–457

Saxelin M (1997) *Lactobacillus* GG—a human probiotic strain with thorough clinical documentation. Food Rev Int 13:293–313

Saxelin B, Grenov U, Svensson R, Fonden R, Reniero T, Mattila-Sandholm T (1999) The technology of probiotics. Trends Food Sci Technol 10:387–392

Schaller-Povolny LA, Smith DE (2001) Viscosity and freezing point of a reduced fat ice cream mix as related to inulin content. Millchwissenschaft 56:25–29

Scheinbach S (1998) Probiotics: functionality and commercial status. Biotechnol Adv 16:581–608

Schmidt KA, Lundy A, Reynolds J, Yee LN (1993) Carbohydrate or protein based fat mimicker effects on ice milk proteins. J Food Sci 58:761–763

Shah NP (2000) Probiotic bacteria: selective enumeration and survival in dairy products. J Dairy Sci 83:894–907

Shah NP (2007) Functional cultures and health benefits. Int Dairy J 17:1262–1277

Shah NP, Ravula R (2000) Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. Aust J Dairy Technol 55:139–144

Shah NP, Ravula R (2004) Selling the cells in desserts. Dairy Ind Int 69:31–32

Sheu TY, Marshall RT (1993) Microencapsulation of Lactobacilli in calcium alginate gels. J Food Sci 54:557–561

Sofjan R, Hartel RW (2004) Effects of overrun on structural and physical characteristics of ice cream. Int Dairy J 14:255–262

Spiegel JE, Rose R, Karabell P, Frankos VH, Schmitt DF (1994) Safety and benefits of fructooligosaccharides as food ingredients. Food Technol 48:85–89

Stanton C, Desmond C, Coakley M, Collins JK, Fitzgerald G, Ross P (2003) Challenges facing development of probiotic-containing functional foods. In: Mazza G (ed) Handbook of fermented functional foods. CRC, Boca Raton, pp 27–58

Steenson LR, Klaenhammer TR, Swaisgood HE (1987) Calcium alginate-immobilized cultures of lactic streptococci are protected from attack by lytic bacteriophage. J Dairy Sci 70:1121–1127

Stone H, Sidel JL (2004) Sensory evaluation practices, 3rd edn. London, Elsevier

Stoon AE (2002) The top 10 functional food trends: the next generation. Food Technol 56:32–37

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int J Food Microbiol 62:47–55

Sunohara H, Ohno T, Shibata N, Seki K (1995) Process for producing capsule and capsule obtained thereby. US Patent 5:478–570

Takahashi N, Xiao JZ, Miyaji K, Iwatsuki K (2007) H^+-ATPase in the acid tolerance of *Bifidobacterium longum*. Milchwissenschaft 62:151–153

Talwalkar AI, Kailasapathy KA (2003) Effect of microencapsulation on oxygen toxicity in probiotic bacteria. Aust J Dairy Technol 58:36–39

Talwalkar AI, Kailasapathy KA (2004) The role of oxygen in the viability of probiotic bacteria with reference to *L. acido*philus and *Bifidobacterium* spp. Curr Issues Intest Microbiol 5:1–8

Talwalkar AI, Miller CW, Kailasapathy K, Nugyen MH (2004) Effect of packaging conditions and dissolved oxygen on the survival probiotic bacteria in yoghurt. Int J Food Sci Technol 39:605–611

Tamime AY, Saarela M, Sondergaard AK, Mistry VV, Shah NP (2005) Production and maintenance of viability of probiotic micro-organisms in dairy products. In: Tamime AY (ed) Probiotic dairy products. Blackwell, Oxford, pp 39–72

Tuorila H, Cardello AV (2002) Consumer responses to an off flavour in juice in the presence of specific health claims. Food Qual Prefer 13:561–569

Turgut T, Cakmakci S (2009) Investigation of the possible use of probiotics in ice cream manufacture. Int J Dairy Technol 62:444–451

Vardar NB, Öksüz Ö (2007) Artisan strawberry ice cream made with supplementation of *Lactococci* or *Lactobacillus acidophilus*. Ital J Food Sci 19:403–411

Vasiljevic T, Shah NP (2008) Probiotics—from Metchnikoff to bioactives. Int Dairy J 18:714–728

Vinderola CG, Bailo N, Reinheimer JA (2000a) Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. Food Res Int 33:97–102

Vinderola CG, Prosello W, Ghiberto D, Reinheimer JA (2000b) Viability of probiotic (*Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei*) and nonprobiotic microflora in Argentinian cheese. J Dairy Sci 83:1905–1911

Wenrong S, Griffiths MW (2000) Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. Int J Food Microbiol 61:17–26

Yackinous C, Wee C, Guinard JX (1999) Internal preference mapping of hedonic ratings for ranch salad dressings varying in fat and garlic flavour. Food Qual Prefer 10:401–409

Ziemer CR, Gibson GR (1998) An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspective and future strategies. Int Dairy J 8:473–479