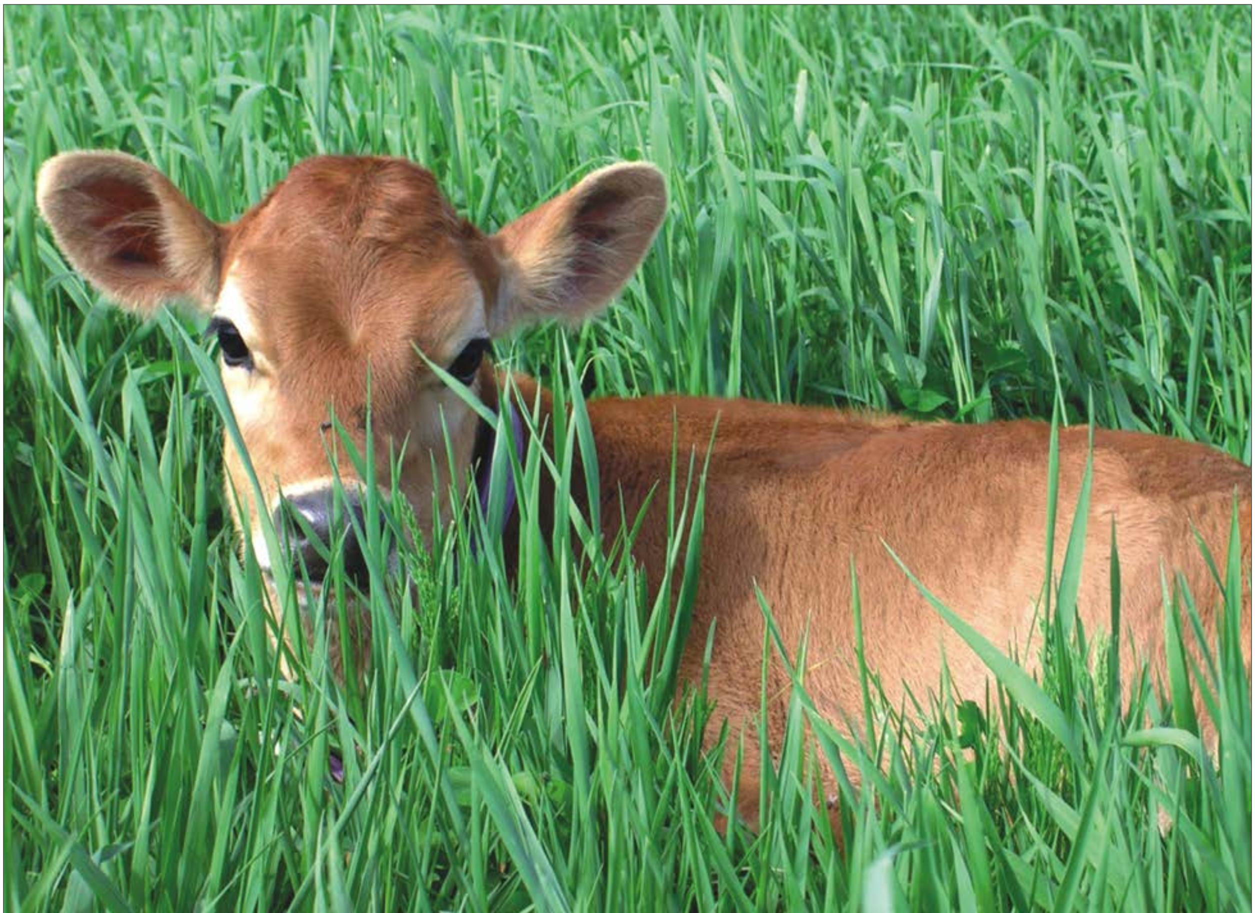

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Factors Influencing Probiotic Survival in Ice Cream: A Review

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ABSTRACT

Among the probiotic dairy products with live probiotics, non-fermented probiotic ice cream is a good vehicle for delivery of live probiotic cells to human intestinal tract because of its neutral pH and high total solids level which provides protection for the probiotic bacteria. On the other hand, harsh conditions of ice cream formulation and manufacturing (high osmotic pressure, high oxygen content in varies overruns and packaging materials, freezing and storage temperatures), acidic and alkaline conditions of human intestinal tract may reversely alter the probiotic survival. This study reviews the factors affecting probiotic survival in ice cream.

Key words: Probiotic, survival, ice cream, sensory properties, development

INTRODUCTION

Due to the large number of microbial cells in the human gut (10 times) in comparison with body cells in adults, the change of microbial balance in human intestine can impress the host health. The ratio between the beneficial microbes (probiotics) and harmful microbes would have an important effect on host health. Therefore, regular consumption of foods containing probiotic cells may keep up the probiotic counts in the human gut. Among dairy probiotic products, ice cream is a good vehicle to transfer probiotics into the human intestinal tract (Mountzouris and Gibson, 2003; Homayouni *et al*., 2008a).

Probiotics are distinct as live micro organisms, when administered in sufficient amounts present a health benefit on the host (FAO United Nations and WHO, 2002; Homayouni, 2009). In recent years probiotic bacteria have increasingly been incorporated into dairy foods as dietary adjuncts. *Lactobacillus* and *Bifidobacterium* are the most common probiotic bacteria that were used in the production of fermented and non-fermented ice cream.

Consumption of probiotic bacteria via dairy food products is an ideal way to re-establish the intestinal micro-flora balance. For a dairy food product to be considered as a valuable alternative for delivery of probiotic bacteria in one hand and for variety of probiotic cultures to use as a dietary adjunct and to exert a positive influence in the other hand, it must conform to certain requirements. The culture must be native of the human gastrointestinal tract, having the ability to ferment prebiotics, survives passage through the stomach and small bowel in adequate numbers, be capable of colonizing in site of action and have beneficial effects on human health. In order to survive, the strain must be resistant to acidic conditions (gastric pH 1-4), alkaline conditions (bile salts present in the small bowel), enzymes present in the intestine (lysozyme) and toxic metabolites produced during digestion. In the case of dairy food product to be considered as a valuable alternative for delivery of probiotics, it must to match definite necessities such as neutral pH, high enough total solids level, absence of oxygen and near to ambient temperatures (Gilliland, 1989; Hoier, 1992; Martin and Chou, 1992; Homayouni *et al.*, 2008b, c). Therefore, development of probiotic frozen dairy products is a key research priority for food design and a challenge for both industry and science sectors. This article presents an overview on probiotic ice cream development.

Probiotic ice cream: Probiotic ice cream can be produced by incorporation of probiotic bacteria in both of fermented and unfermented mix. Ice cream is ideal vehicle for delivery of these organisms in the human diet (Akin *et al.*, 2007; Hekmat and McMahon, 1992; Kailasapathy and Sultana, 2003; Ravula and Shah, 1998a). *Lactobacillus* and *Bifidobacterium* are the most common species of lactic acid bacteria used as probiotics for fermented dairy products. Among the frozen dairy products with live probiotics, probiotic ice cream is also gaining popularity for its neutral pH. The pH of non-fermented ice cream is near to seven which is providing to survive probiotic bacteria. (Christiansen *et al.*, 1996; Akin *et al.*, 2007; Homayouni *et al.*, 2008c). The high total solids level in ice cream including the fat and milk solids provides protection for the probiotic bacteria. Because the efficiency of added probiotic bacteria depends on dose level, type of dairy foods, presence of air and low temperature (Homayouni *et al.*, 2008a), their viability must be maintained throughout the product's shelf-life and they must survive the gut environment (Kailasapathy and Chin, 2000).
The therapeutic value of live probiotic bacteria is more than unviable cells; therefore, International Dairy Federation (IDF) recommends that a minimum of 10^7 probiotic bacterial cells should be alive at the time of consumption per gram of product. Studies indicate, however, the bacteria may not survive in high enough numbers when incorporated into frozen dairy products unless a suitable method is used against freeze injury and oxygen toxicity (Dave and Shah, 1998; Hekmat and McMahon, 1992; Kailasapathy and Sultana, 2003; Ravula and Shah, 1998a). Figure 1 represents factors affecting probiotic survival in ice cream.

The methods of increasing probiotic survival depend on type of food products. Selection of resistant probiotic strains to tolerate production, storage and gastrointestinal tract conditions, is one

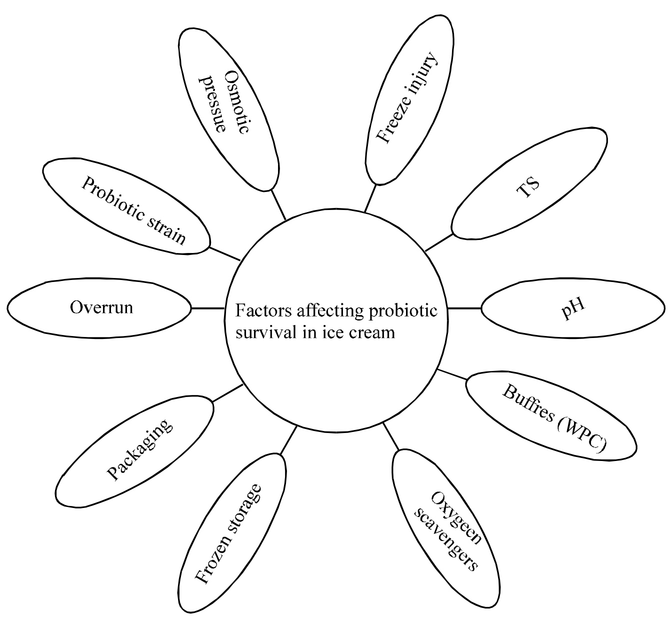

Fig. 1: Factors affecting probiotic survival in ice cream

of the important methods. Another way is to adjust the conditions of production and storage for more survival rates. The physical protection of probiotics by microencapsulation is a new method for increasing the survival of probiotics. Encapsulation helps to isolate the bacterial cells from the adverse environment of the product and gastrointestinal tract, thus potentially reducing cell loss. Encapsulation thus may enhance the shelf-life of probiotic cultures in frozen dairy products (Kebary *et al*., 1998; Shah and Ravula, 2000; Homayouni *et al*., 2008c). Selecting of suitable probiotic strains depends to ability survive simulated conditions of ice cream (high sucrose concentrations, high oxygen, freezing and storage temperatures), acidic (to simulate gastric) and alkaline conditions (to simulate intestinal). Microencapsulation of probiotics can further protect these bacteria from the mentioned conditions.

Selection of appropriate probiotic strains for use in probiotic ice cream: During the recent two decades, several studies have shown that the ice cream has a good ability for distribution of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* into human gut. Frozen dairy products create ideal conditions for probiotic bacteria to survive for a long-term of production, distribution and storage (Hekmat and McMahon, 1992). Ice cream provides good conditions for probiotic growth in large numbers and their survival during storage. Low-fat ice cream in comparison with regular one provides better conditions for the survival of *Lactobacillus acidophilus*, *Lactobacillus paracasei* and *Bifidobacterium lactis* (Mizota 1996). Dairy frozen desserts which are prepared from yoghurt, may decrease the bio-availability of *Lactobacillus acidophilus* and *Bifidobacterium* species because of low pH<4.5 (Ravula and Shah, 1998b), while incorporation of probiotic bacteria in non-fermentative ice cream does not create any problem because ice cream pH (6.6-6.5) is ideal for probiotics survival.

During the 11 weeks of storage in fermented low-fat ice cream and frozen yoghurt no change was observed in the primer number of probiotic cells (Gomes and Malcata, 1999). Also there is no change was occurred in amount of protein, lactose and sensory characteristics (Davidson *et al*., 2000). Survival of *Lactobacillus johnsonii* in ice cream with two levels of sucrose and fat in two different temperature showed that the number of bacterial cells were not decreased within eight months of cold storage (Alamprese *et al*., 2002). Other study was showed that the number of *Lactobacillus rhamnosus* (GG) in ice cream with two levels of sucrose (15 and 22%) and fat (5 and 10%) over one year storage at different temperatures (-16 and -28°C) does not changed (Alamprese *et al*., 2005). Also after six months storage of ice cream containing sucrose and aspartame, at -20°C, the number of *Lactobacillus acidophilus*, *Lactobacillus agilis* and *Lactobacillus rhamnosus*) does not changed significantly (Basyigit *et al*., 2006).

It was shown that *Bifidobacterium bifidum* and *Bifidobacterium lactis* in fermented ice cream with yoghurt starter culture enriched with vitamin C do not changed significantly over 15 weeks at -18°C (Favaro-Trindade *et al*., 2006). In ice cream containing 4% of fat in its formulation, 86-90% of the *Lactobacillus acidophilus* and *Bifidobacterium lactis* were survived during 2 months of storage at -25°C (Magarinos *et al*., 2007). It was also demonstrated that inulin can increase the survival of *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream (Akin *et al*., 2007). On the other hand the sensory analysis results were shown that the taste of ice cream fermented by yoghurt starter culture and/or probiotic bacteria was less accepted in comparison with non-fermentative ice cream (Favaro-Trindade *et al*., 2007).

In recent studies it was showed that it is possible to better adapt the probiotic strains to the conditions of probiotic ice cream. Magarinos *et al*. (2007) have demonstrated that a large decrease in probiotic counts was occurred during dilution of probiotic culture with addition into ice cream mixture and incorporation of air in the freezing process. In another study, the production of probiotic ice cream with different amounts of inulin had significant effect on probiotic counts (Akin *et al.*, 2007). Also the casein hydrolysate or cysteine has a protective effect for *B. lactis* (Ravula and Shah, 1998b). So, the probiotics stability in probiotic ice cream is dependent on species and strain and survival of probiotics during ice cream storage appears to depend on bacterial species/strain and ice cream composition.

Presence of the prebiotic inulin appears to support survival of probiotic strains of *Lactobacillus* and *Bifidobacterium* during frozen storage, as do casein hydrolysate and cysteine. Bacterial cells from different growth phases may be differently sensitive to the conditions of ice cream manufacture. Probiotic cell harvesting in the beginning of stationary-phase, may cause them to be metabolically less active, less susceptible to stress and in high cell counts. Carbon starvation, cold shock and/or oxidative stress prior to ice cream manufacture may favor survival of probiotics (Wetzel *et al.*, 1999). Enhanced expression of certain proteins such as betaine transporter (BetL) may enhance survival of probiotics in ice cream. Candidate proteins are: Dpr, SodA, BetL. Expression of betaine transporter (BetL) enhances survival following repeated freeze/thaw cycles at -20°C (Sheehan *et al.*, 2006). Expression of cold shock protein CspP enhances survival following repeated freeze/thaw cycles at -80°C (Derzelle *et al.*, 2003). Enhanced expression of superoxide dismutase (SodA) protects *L. gasseri* and *L. acidophilus* against oxygen (Bruno-Barcena *et al.*, 2004). During ice cream manufacture probiotics will be exposed to certain stresses, namely freezing stress and oxidative stress. Some strains however are already sufficiently stable by exerting peroxide resistance protein (Dpr), general stress protein, chaperonins ClpL and GroEL (Arena *et al.*, 2006). Production and harvesting of probiotics may considerably affect their probiotic potential in ice cream. Probiotics not only have to be present in certain numbers, but they have to be metabolically active at the site of action. Enhanced expression of certain proteins-e.g. stress proteins -may enhance survival of probiotics in ice cream. Exposure to carbon starvation, cold shock and/or oxidative stress prior to ice cream manufacture may favor survival of probiotics. Stability of probiotics in ice cream may be enhanced by changing the ice cream mixture.

In order to select appropriate probiotic strains for use in probiotic ice cream a study was conducted by Homayouni (2008) in simulated ice cream and gastrointestinal conditions (Homayouni *et al.*, 2008c). The growth and survival rate of *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium lactis* and *Bifidobacterium longum* in varying amount of sucrose concentrations (10, 15, 20 and 25%), oxygen scavengering components (0.05% L-cysteine and 0.05% L-ascorbate) and low temperatures (4 and -20°C) during different periods of time (1, 2 and 3 months) in MRS-broth medium was studied by Homayouni *et al.* (2008b). All of the stress factors examined have been able to influence the growth and survival of related probiotics. The results have demonstrated that it is possible to select the suitable probiotic strains for use in probiotic ice cream. In summary, a comparison with other probiotic strains revealed that *Lactobacillus casei* (*Lc01*) and *Bifidobacterium lactis* (*Bb12*) had the highest resistance to simulated acidic, alkaline and ice cream conditions, making them suitable probiotic strains for use in probiotic ice cream (Homayouni *et al.*, 2008b,c).

Microencapsulation of probiotic strains for increasing probiotic survival: Microencapsulation is defined as technology of tiny liquid or solid particles packaging (Homayouni, 2008). The effect of microencapsulation on probiotic survival in various food products is a new subject in recent two decades. The addition of microencapsulated *Lactobacillus acidophilus* and

Bifidobacterium spp. to yoghurt can increase the survival of these bacteria 0.5-1 log cycle (Sultana *et al.*, 2000). Using the two-step microencapsulation for Bifidobacteria in alginate and poly-L-lysine can protect these probiotics against cold (4°C) and acidic conditions (Cui *et al.*, 2000). Application of 10% (by weight) of gelatin, Arabic gum and starch as coating materials, increases the survival of *Bifidobacterium* spp. in spray drying process. When *Bifidobacterium longum* is microencapsulated in skim milk, the highest rate of survival can be achieved (Lian *et al.*, 2002). Microencapsulation may increase the survival of *Bifidobacterium longum* in refrigerated milk (2% fat) more than free cells but do not protect *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis* and *Bifidobacterium breve* against acidic conditions (pH 2 and 3) and alkali (5 and 10 g L^{-1} of bile salts) (Hansen *et al.*, 2002). The probiotic bacteria stay alive against oxygen when the alginate microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* were added to the yoghurt (Talwalkar and Kailasapathy, 2003). Microencapsulation of *Bifidobacterium breve* in whey proteins coating with spray drying method, results in increased survival of this bacterium in the yoghurt during 28 days storage at 4°C (Picot and Lacroix, 2004). Microencapsulation of *Lactobacillus acidophilus* and *Lactobacillus casei* in beads containing two layers of alginate and chitosan, can protect these bacteria against the acidic conditions (pH 1.5) and subsequent alkaline conditions (0.6 percent bile salts) (Krasaekoopt *et al.*, 2004). Application of skim milk as a coating material for the microencapsulation of *Bifidobacterium spp.* can increase survival during spray drying (Simpson *et al.*, 2005). The increasing of alginate concentration from 2 to 4 percent can increase the survival of *Lactobacillus casei* against acidic conditions (pH 1.5), alkaline (1 and 2 percent bile salts) and high temperatures (55, 60 and 65°C) (Mandal *et al.*, 2006). Addition of microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* to the yoghurt can increase the survival of these bacteria, respectively, 1 and 2 logarithmic cycle in comparison with free cells without any changes in color, flavor and texture of yoghurt. Also, postpones the secondary acidification of yoghurt during its shelf life storage (Kailasapathy, 2006). In fermented sausage the addition of microencapsulated *Lactobacillus reuteri* and *Bifidobacterium longum*, can increase survival of these probiotics but reduces resistance effect against the Escherichia coli. It may be the result of low permeability characteristics of bead wall against antimicrobial compounds (Muthukumarasamy and Holley, 2007). In conclusion, microencapsulation is a good way to preserve microbial cells alive during the production process of functional foods and delivery of viable bacteria to intestine (Anal and Singh, 2007).

In resent years, there has been a lot of interest to use microencapsulation technique for increasing the survival of probiotic cells in ice cream and frozen dairy products (Table 1). The physical protection of probiotics by microencapsulation is a new approach to improve the probiotic survival. Encapsulation helps to isolate the bacterial cells from the harsh environment and gastrointestinal tract, thus potentially prevents cell loss. To some extent, Kebary *et al.* (1998)

Table 1: Encapsulation of lactic acid bacteria by emulsion technique for use in ice cream and frozen desserts

Probiotic bacteria	Bead size (diameter)	Application	Reference
Lactobacillus delbrueckii spp. *bulgaricus*	-	ice cream	Sheu and Marshall (1991)
Lactobacillus delbrueckii spp. *bulgaricus*	30 μm	frozen dessert	Sheu *et al.* (1993)
Lactobacillus delbrueckii spp. *bulgaricus*	25-35 μm	frozen ice milk	Sheu and Marshall (1993)
Bifidobacterium bifidum Bifidobacterium infantis	-	ice milk	Kebary *et al.* (1998)
Lactobacillus acidophilus Bifidobacterium lactis	0.5-1 mm	ice cream	Kailasapathy and Sultana (2003)
Lactobacillus casei *Bifidobacterium lactis*	10-30 μm	ice cream	Homayouni *et al.* (2008b)

have shown that *Bifidobacterium spp.* survive in high numbers in frozen ice milk in beads made from alginate than those made from k-carrageenan. Shah and Ravula (2000) reported that the survival of probiotic bacteria in fermented frozen desserts improved with encapsulation. Encapsulation thus may enhance shelf-life of probiotic cultures in frozen dairy products (Kebary *et al.*, 1998; Shah and Ravula, 2000; Homayouni *et al.*, 2007).

Encapsulation can significantly improve the survival of probiotic bacteria in symbiotic ice cream (Homayouni *et al.*, 2008b). Survival of free and microencapsulated *L. casei* (*Lc01*) and *B. lactis* (*Bb12*) in synbiotic ice cream containing resistant starch as a prebiotic substance was investigated by Homayouni (2008b). This study was carried out to evaluate incorporating possibility of resistant starch into bead coating and ice cream formulation. After one month, microencapsulated probiotics in alginate beads were survived 30% more than uncapsulated bacteria. The numbers of viable probiotic bacteria in all types of ice cream were between 10^8-10^9 CFU g^{-1} after three months, higher than that recommended by the International Dairy Federation (10^7 CFU g^{-1}). These results showed that the high initial number of encapsulated probiotics can provide the required standard in the probiotic ice cream (Homayouni *et al.*, 2008b).

Design of probiotic ice cream: Development of probiotic ice cream requires detailed knowledge of both product and customers. In the other hand, it needs to manage customer knowledge effectively. From a research and development point of view, functional probiotic ice cream represents an area where the expertise of food technologists, nutritionists, medical doctors and food chemists must be combined to obtain innovative products. In addition, these products must be able to modulate physiological parameters related to health status or disease prevention. The design and development of functional probiotic ice cream is an expensive and multistage process that takes into account many factors, such as sensory acceptance, physicochemical and microbial stability, price and other intrinsic functional properties to be successful in the marketplace. Moreover, consumer expectation toward the functional probiotic product also needs to be taken into consideration (Walzem, 2004; Fogliano and Vitaglione, 2005; Jousse, 2008).

Sensory properties of probiotic ice cream: Probiotic ice cream can create different flavor profiles when compared with conventional one. Inulin and Oligofructose provide some suitable sensory properties to ice cream in which it is added, such as rounder mouthfeel, sustained of flavor with reduced aftertaste and slight sweetness. These properties are partly responsible for high score values for taste, creaminess and acceptability of synbiotic ice cream. Flavor is first indicator with respect to a food choice, followed by considerations regarding health benefits. If the ingredients added contribute unpleasant flavors to the product, consumers are not interested in consuming a functional probiotic ice cream even if this results in advantages with respect to their health. When functional ingredients such as probiotics are added to ice cream, consumers must be aware of probiotics health benefits in order to persuade that the functional probiotic ice cream is being more beneficial than the traditional one. How to communicate the beneficial effects on health in a way understandable to all consumers is one of the most important aspects of developing new probiotic ice cream (Tepper and Trail, 1998; Mattila-Sandholm *et al.*, 1999; Roberfroid, 2000; Tuorila and Cardello, 2002; Nicolay, 2003; Vieira, 2003).

The overall acceptability of non-fermented probiotic ice cream is the same as conventional one but in fermented probiotic ice cream as well as fermented frozen desserts, low pH values (4.0 to 4.5) has negative effects on sensory acceptance, since ice cream is not traditionally characterized as an acidic food product. However, an increase in sugar concentration can improve sensory properties but adding inulin had no effect on it. The addition of *L. paracasei* subsp. *paracasei* and inulin did not interfere and even improved the sensory preference of the mousse (Akin *et al.*, 2007; Aragon-Alegro *et al.*, 2007; Homayouni *et al.*, 2008b; Cruz *et al.*, 2009).

CONCLUSIONS

The future success of functional probiotic dairy foods in marketplace depends on consumer acceptance of such products. Development of probiotic dairy products is a key research priority for food design and a challenge for both industry and science sectors. Among the functional foods, the dairy probiotic products, especially ice cream and cheese are good vehicle to transfer probiotics to the human intestinal tract. Additional way to keeping up the probiotic cells in the gut is to entering prebiotics into the intestine through the regular consumption of food containing these components. More studies are needed to further investigate the probiotic survival in harsh conditions of ice cream formulation and manufacturing (high osmotic pressure, high oxygen content in varies overruns and packaging materials, freezing and storage temperatures), acidic and alkaline conditions of human intestinal tract as well as therapeutical effects of live probiotic cells on human health.

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