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Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream

A. Homayouni [a,*], A. Azizi [b], M.R. Ehsani [c], M.S. Yarmand [c], S.H. Razavi [c]

[a] Department of Food Science and Technology, Faculty of Health and Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Iranian Agricultural Engineering Research Institute, Ministry of Agriculture, Tehran, Iran
[c] Department of Food Science and Technology and Technology, Faculty of Biosystem Engineering, Campus of Agriculture and Natural Resources, The University of Tehran, Tehran, Iran

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ABSTRACT

Two types of synbiotic ice cream containing 1% of resistant starch with free and encapsulated *Lactobacillus casei (Lc-01)* and *Bifidobacterium lactis (Bb-12)* were manufactured. The survival of *L. casei* and *B. lactis* were monitored during the product's storage for 180 days at −20 °C. The viable cell number of *L. casei* and *B. lactis* in the free state in prepared ice cream mixture was 5.1×10^9 and 4.1×10^9 CFU/mL at day one and after 180 days storage at −20 °C, these numbers were decreased to 4.2×10^6 and 1.1×10^7 CFU/mL, respectively. When we encapsulated the mentioned probiotic bacteria in calcium alginate beads, the probiotic survival raised at rate of 30% during the same period of storage at same temperature. In general, the results indicated that encapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended shelf-life. The addition of encapsulated probiotics had no significant effect on the sensory properties of non-fermented ice cream in which we used the resistant starch as prebiotic compound.

1. Introduction

Probiotics are defined as live microorganisms which, when administered in adequate amounts confer a health benefit to the consumers. Prebiotics are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (FAO/WHO, 2001). A food product containing both probiotics and prebiotics is named as synbiotic or functional food (Homayouni, Ehsani, Azizi, Razavi, & Yarmand, 2007a). There is a synergy between probiotics and prebiotics in synbiotic products. Prebiotic compounds are consumed by probiotics as a carbon or energy source in the colon. This results in an increase in the probiotic count and the reduction of pathogen microorganisms in the gut (Vernazza, Rabiu, & Gibson, 2006).

Probiotic bacteria have been incorporated into fermented and non-fermented ice cream which is an ideal vehicle for delivery of these organisms in the human diet (Akin, Akin, & Kirmaci, 2007; Hekmat & McMahon, 1992; Kailasapathy & Sultana, 2003; Ravula & Shah, 1998). Due to its neutral pH, synbiotic ice cream is also gaining popularity (Akin et al., 2007). The pH of non-fermented ice cream is closer to 7 and this provides possibility for satisfactory survival of probiotic bacteria (Christiansen, Edelsten, Kristiansen, & Nielsen, 1996). The efficiency of added probiotic bacteria depends on the dose level, temperature, type of dairy foods and presence of air (Homayouni, Ehsani, Azizi, Yarmand, & Razavi, 2006a), their viability must be maintained throughout the product's shelf-life and the gut environment (Kailasapathy & Chin, 2000). The therapeutic value of probiotic bacteria normally depends on the viability of these bacteria. Therefore, International Dairy Federation (IDF) has suggested that a minimum of 10^7 probiotic bacterial cells should be alive at the time of consumption per gram of the product. Some authors have shown that the freezing process affects dramatically the number of live probiotic cells (Dave & Shah, 1998; Hekmat & McMahon 1992; Kailasapathy & Sultana, 2003; Ravula & Shah 1998). We suggest that the loss of viability of probiotic organisms in frozen dairy desserts is due to the effect of freezing operation on the cell wall or the oxygen toxicity.

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 effects of the hostile environment and gastrointestinal tract, thus potentially preventing cell loss. To some extent, Kebary, Hussein, and Badawi (1998) 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 the shelf-life of probiotic cultures in frozen dairy products.

* Corresponding author. Tel.: +98 411 3357581; fax: +98 411 3340634.
E-mail addresses: Homayouni@ut.ac.ir, Homayounia@tbzmed.ac.ir (A. Homayouni).

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It was reported that ice cream can serve as a good carrier for delivering probiotic bacteria to consumers (Akin et al., 2007; Haynes & Playne 2002; Hekmat & McMahon 1992; Kailasapathy & Sultana, 2003), however, survival of free and microencapsulated *Lactobacillus casei (Lc-01)* and *Bifidobacterium lactis (Bb-12)* in synbiotic ice cream containing resistant starch as a prebiotic substance has not yet been reported. Furthermore, no reports were found in the literature about the sensory properties of such product. Therefore the objectives of this study were to evaluate the feasibility of incorporating resistant starch into bead coating and icecream formulation and to investigate the survival of microencapsulated and free cultures in ice cream over a period of 180 days storage at −20 °C.

2. Materials and methods

2.1. Cultures and enumeration of free and encapsulated probiotics

Pure freeze-dried probiotic culture of *L. casei (Lc-01)* and *B. lactis (Bb-12)* were obtained from CHR-Hansen (Horsholm, Denmark) and were activated by inoculating in the MRS-broth (de Man-Rogasa-Sharpe) at 37 °C for 24 h. The probiotic biomass in late-log phase was harvested by centrifugation at 600*g* for 10 min at 4 °C (Sorvall, model RC-5C, rotor GS-3, Newtown, CT), then washed twice in sterile 0.9% saline under the same centrifugation conditions, and used in the microencapsulation process. Bacterial counts were determined before freezing and immediately after freezing as well as at the end of every 30 days until 180 days of storage at −20 °C. The samples of ice cream mixture prior and after freezing (10 g) were decimally diluted in 100 mL sterile peptone water (0.1%) and 1 mL aliquot dilutions were poured onto plates of the MRS-agar in triplicate. Enumeration of probiotic bacteria was achieved as described by Haynes and Playne (2002). A different container of ice cream was sampled on each day of enumeration. All enumerating plates of *L. casei* and *B. lactis* were incubated at 37 °C for 72 h under aerobic and anaerobic conditions, respectively. The averages of all results were expressed as colony-forming units per gram of sample (CFU g^{-1}). To count the encapsulated bacteria in ice cream, the entrapped bacteria were released from the beads according to the method of Sheu and Marshall (1993). Ten grams of ice cream were re-suspended in 100 mL of phosphate buffer (0.1 M, pH 7.0), followed by shaking in a stomacher for 10 min. The ice cream sample containing free bacteria was treated in a similar way, in order to maintain the same treatment conditions. The counts (CFU g^{-1}) were determined by plating on MRS-agar as discussed above.

2.2. Encapsulation procedure

All glasswares and solutions used in the protocols were sterilized at 121 °C for 15 min. Alginate beads were produced using a modified encapsulation method originally reported by Sheu and Marshall (1993) and Sultana et al. (2000). A 2% alginate mixture in distilled water was prepared containing 2% Hi-maize resistant starch (Merck, Darmstadt, Germany) and 0.1% culture (18 h old culture grown in MRS-broth). The mixture was added into 200 mL canola oil (Ladan, Behshahr Oil Industry, Behshahr, Iran) containing 0.2% lecithin (Nazgol Oil Industry, Mahidasht, Kermanshah, Iran). The mixture was stirred vigorously (400 rpm for 20 min, Heydolph Stirrer, Germany) until it was fully emulsified. Then 200 mL of 0.1 M calcium chloride solution were added. The mixture was allowed to stand 30 min to separate prepared calcium-alginate beads in the bottom of beaker at the calcium chloride layer. The oil layer was drained and beads were collected in the calcium chloride solution which was then washed with 0.9% saline containing 5% glycerol and stored at 4 °C.

2.3. Acidification kinetics of microencapsulated bacteria

Free and encapsulated cells of *L. casei* and *B. lactis* were cultivated in MRS-broth to determine if the encapsulated cells were still metabolically active and if nutrients and metabolites could permeate bead wall. To obtain the time needed for free and encapsulated cells to acidify MRS-broth medium, $(2.5 \pm 0.1) \times 10^6$ (CFU g^{-1}) of free cells and $(2.5 \pm 0.3) \times 10^6$ (CFU g^{-1}) of encapsulated cells (in beads) were inoculated into the 100 mL MRS-broth medium and incubated at 37 °C for 56 h. The pH values and optical density (OD) at 525.2 nm were measured every 4 h (Homayouni, Ehsani, Azizi, Razavi, & Yarmand, 2008b).

2.4. Ice cream – making procedure

Cow's milk (55.4%) and heavy cream (20%) were supplied from the animal husbandry unit of the Campus of Agricultural and Natural Resources of the University of Tehran, Karaj. The dry matter content in the milk was adjusted by adding skim milk powder (6%; Pegah Dairy Industries Co., Tehran, Iran), sugar (17%; Sugar Industries Co., Ahvaz, Iran) and resistant starch (1%; Merck, Darmstadt, Germany). FO41 (0.5%; PROVISCO, Hauptwil, Switzerland) containing mono- and diacylglycerols (E 471), locust bean gum (E 410), guar gum (E 412), carrageenan (E 407) was used as stabilizers and vanillin (0.1%) was added for aroma development. Ice cream was formulated based on 38–39% total solids for a batch of 100 kg.

The milk and cream (40% fat) were mixed and temperature was increased to 50 °C; the blend of skim milk powder, resistant starch and stabilizer along with sugar were added. The resultant mixture was flavoured with vanillin and homogenized in a two-stage homogenizer at 3000 and 500 psi (MFG Company, Chicago, IL) and then pasteurized at 80 °C for 20 s and after pasteurization, the mixture was cooled to 4 °C and stored for 12 h for ripening at this temperature. The ice cream mixture was divided into five parts of 20 L (A, B, C, D and E). 1% (w/v) free *L. casei* and *B. lactis* were added to batches A and B, respectively. The initial counts of these bacteria in ice cream for A and B were about $(9.4 \pm 0.2) \times 10^9$ and $(8.2 \pm 0.3) \times 10^9$ (CFU g^{-1}), respectively. C and D portions of non-fermented ice cream were mixed with freshly prepared encapsulated *L. casei* and *B. lactis*. The initial counts of these bacteria for C and D were about $(5.8 \pm 0.2) \times 10^9$ and $(6.1 \pm 0.5) \times 10^9$ (CFU g^{-1}), respectively. The synbiotic ice cream was produced immediately after the addition of probiotics to the mixture by using a vertical freezing machine of 20 kg capacity (Thompson Machine and Supply Co., Chicago, IL). The partially frozen mixture was packaged in 200 mL cups and stored at −20 °C. The manufacturing procedure for the synbiotic ice cream is schematically shown in Fig. 1. The experiment was conducted in triplicate.

2.5. Chemical and physical analysis

The pH of the ice cream was measured using a digital pH-meter (microprocessor pH-meter, model pH 537, WTW, Weilheim, Germany). Titratable acidity was determined according to Dornic method (ISIRI number 2450, 2005). The dry matters of ice cream were determined by drying samples at 100 ± 1 °C for 5.0 h using an air oven (AOAC, 1997). The fat contents of milk and ice cream were determined using the Gerber method (ISIRI number 2450, 2005). All chemical measurements were done in triplicate. The overrun of the final product was determined using the following formula (Homayouni, Ehsani, Mousavi, Valizadeh, & Djome, 2005,2006b):

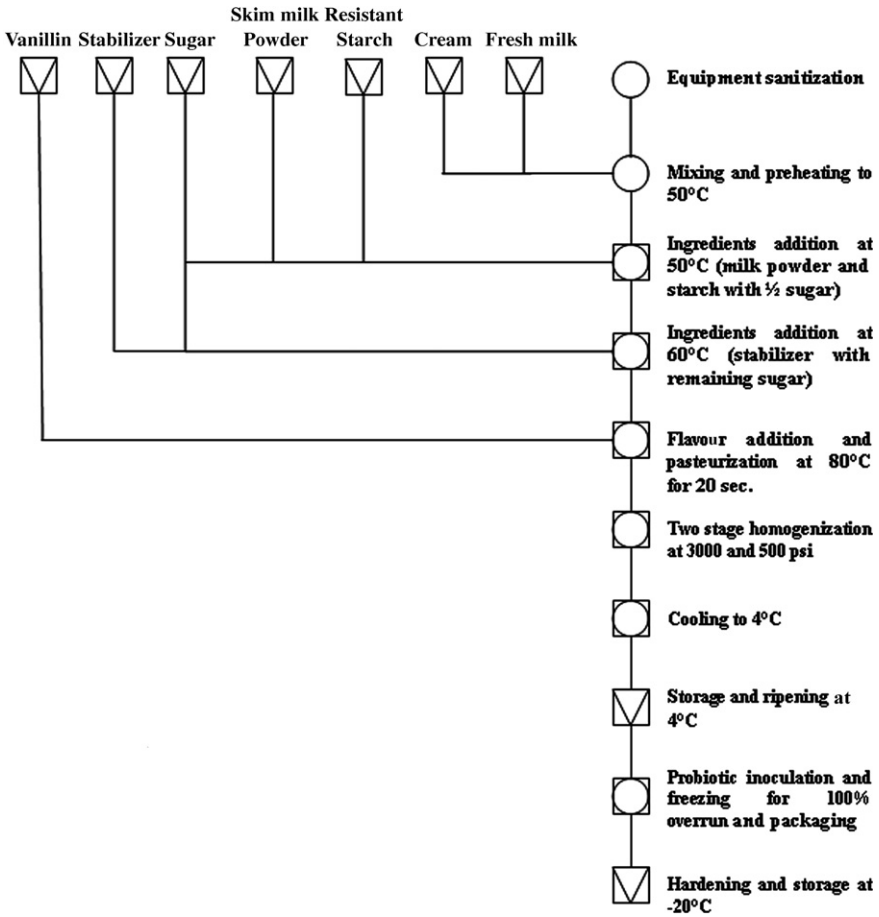

Fig. 1. Schematic representation of the manufacturing procedure for synbiotic ice cream in this study.

$$\text{Overrun} = \frac{\text{Weight of unit mix-weight of equal volume of ice cream}}{\text{Weight of equal volume of ice cream}} \times 100$$

In this study, the size distribution of the microcapsules was measured by scanning electron microscopy (SEM) and also by optical microscopy (Carlzeiss, Jena, Teichgraben, Germany). The diameter of 120 randomly selected microcapsules were measured for this purpose (Krasaekoopt, Bhandari, & Deeth, 2004; Wojtas, Hansen, & Paulson, 2008). The beads were coated with gold (Sputter Coater, Model SCDOOS, Bal-Tec, Balzers, Liechtenstein, Switzerland) and examined at an accelerating voltage of 20.0 kV. Micrographs were modified by ACD photo editor software, version 3.1 (Software Spectrum UK Ltd., High Wycombe, UK) to isolate the important details from the background (Russ, 2005) by increasing the contrast to more than 95% and brightening the picture with gamma adjustment. The Microstructure Measurement Software (MMS, Ferdowsi University, Mashhad, Iran) was then used to measure the bead size in the randomly selected beads.

2.6. Sensory analysis

The synbiotic ice cream samples were organoleptically assessed by 32 panelists under fluorescent white light using a sensory rating scale of 1–10 for flavour and taste, and 1–5 for body and texture and 1–5 for colour and appearance, as described by Homayouni, Ehsani, Mousavi, Valizadeh, & Djome, 2006b. The properties evaluated included the following score ratings: (a) eight attributes for flavour and taste (no criticism: 10, cooked flavour: 9–7, lack of sweetness and too sweet: 9–7, lack of flavour: 9–6, yogurt/probiotic flavour: 8–6, acidic/sour: 8–6, rancid and oxidized: 6–1, and other: 5–1), (b) seven characteristics of body and texture (no criticism: 5, crumbly: 4–2, coarse: 4–1, weak: 4–1, gummy: 4–1, fluffy: 3–1, sandy: 2–1) and (c) four terms describing colour and appearance (no criticism: 5, pale colour: 4–1, non-uniform colour: 4–1, unnatural colour: 3–1). The panel of assessors was an external panel of non-smokers who were trained for dairy products sensory evaluations and were checked on the basis of sensory acuity and consistency by testing their accuracy to recognize four principle tastes. Samples were stored at −18 °C in a freezer to maintain integrity during sensory analysis. The consumer acceptance tests were conducted for evaluation of flavour and taste, body and texture, colour and appearance, and overall acceptance (Meilgaard, Civille, & Carr, 1999). Physical, chemical and sensory analyses were carried out one week after production.

2.7. Statistical analysis

The collected data was analyzed by SAS statistics software, Version 6.12 edition (SAS, 1996). The mean values and the standard error were calculated from the data obtained with triplicate trials. These data were then compared by the Duncan's multiple range method.

3. Results and discussion

3.1. Metabolic activity of microencapsulated bacteria

The viability and metabolic activity of microencapsulated probiotic bacteria can be monitored by determination of pH (Sultana et al., 2000) and optical density (Homayouni, Ehsani, Azizi, Yar-

mand, & Razavi, 2007b) of broth medium. Acidification kinetics and optical density ($OD_{525.2}$) over a period of 56 h in MRS-broth medium containing free and encapsulated cells of *L. casei* and *B. lactis* were compared to show that if the encapsulated bacterial cells are active and could produce acid similar to free cells. The results showed that free and encapsulated cell changed the pH value and optical density of MRS broth medium with time, indicating that bacterial cells were remained viable and active in the Calcium alginate beads. However the time taken for the encapsulated cells to arrive at the same pH was longer than that taken by the free cells. The time taken for free cells to decrease the initial pH value of MRS broth medium to four was about 20 h whereas that of encapsulated cells was about 50 h. This could be due to the slow uptake of nutrients and slow release of the metabolites across the encapsulating alginate shell of beads (Homayouni et al., 2007b). A similar pattern was also observed by Larisch, Poncelet, Champagne, and Neufeld (1994) in alginate/poly-L-lysine beads containing Lactococci cells. It has been suggested that the size of the beads affects the rate of mass transfer and metabolic activity of microencapsulated cells.

3.2. Chemical and physical characteristics

The chemical composition of the cow milk used in the production of synbiotic ice cream was: pH 6.60 ± 0.01, titratable acidity 6.55 ± 0.02°SH, dry matter 12.09 ± 0.05% and fat 3.20 ± 0.10%. The dry matter and fat content of the ice cream mixture was: 38.50 ± 0.15% and 8.10 ± 0.05%, respectively. The overrun value was 95 ± 5.0. The shape of beads was spherical and their mean diameter was 17.80 ± 3.55 μm (Homayouni et al., 2007b). Sheu, Marshall, and Heymann (1993) reported that a mean diameter of 30 μm was desirable for use in frozen dairy desserts. Large beads might cause coarseness of texture in ice milk, whereas small beads did not provide sufficient protection of the bacteria.

3.3. Survival of free and encapsulated bacteria in ice cream

Figs. 2 and 3 show the bacterial counts (*L. casei* and *B. lactis*) in the mixture and synbiotic ice cream. In the case of free *L. casei*, the cell numbers dropped substantially (about 3.4 log numbers) by 180 days of storage at −20 °C. The *B. lactis* count showed an average 2.9 log decreases for the free state after 180 days, while the encapsulated state of the same strains showed a decrease of 1.4 and 0.7 log, respectively. The loss of *L. casei* and *B. lactis* showed significant differences ($P < 0.05$) between the free and encapsulated states in synbiotic ice cream at the end of 180 days frozen storage. Shah and Ravula (2000) reported that microencapsulation improved the counts of *Lactobacillus acidophilus MJLA1* and *Bifidobacterium* spp. *BDBB2* compared to free cells in frozen fermented dairy desserts stored for 12 weeks. A reduction in the counts of culture bacteria in ice cream after freezing has been reported (Kailasapathy & Sultana, 2003). In frozen ice milk, 40% more lactobacilli survived when they were entrapped in calcium alginate beads (Sheu & Marshall, 1993). Most strains of bifidobacteria are sensitive to pH below 4.6 (Lourence & Viljoen, 2002). Therefore, the neutral pH of the non-fermented synbiotic ice cream can prevent the decline of bifidobacteria populations. Since ice cream is a whipped product, oxygen is incorporated in large amounts and *Bifidobacterium* spp. are strictly anaerobic, therefore, oxygen toxicity is a major factor of cell death. The decline in bacterial counts, as a result of freezing, is likely due to the freeze injury of cells, leading eventually to the death of cells. However, the mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mixture may have resulted in a further decrease in bacterial count. Homayouni, Ehsani, Azizi, Razavi, and Yarmand (2008a), Homayouni, Ehsani, Azizi, Yarmand, and Razavi (2007c) have shown that *L. casei* and *B. lactis* were the most resistant strains in simulated ice cream conditions. The results of our study show that the survival of bacteria against unfavorable conditions such as oxygen toxicity or freezing, and storage at lower temperatures in ice cream, is species dependent. This finding is in agreement with those of Haynes and Playne (2002) and Kailasapathy & Sultana, 2003. The survivability of the probiotics, *L. casei*, and *B. lactis*, was expressed as the survival value (S-value), which is the time required destroying 90% or one log cycle of the organism. The S-values of free cells and microencapsulated probiotics in both uncoated and coated beads in ice cream during 180 days storage at −20 °C are shown in Table 1. Comparison of S-value after 30 and 180 days revealed that freezing process had significant ($P < 0.05$) effect on the viability of free cells. In addition, it was demonstrated that, encapsulated cells required longer time to decrease one log cycle in viable counts. Therefore, microencapsulation of probiotic bacteria in beads with diameter about 20 μm can increase the viability of probiotics.

When probiotics were added to ice cream mixture which was then frozen in a continuous ice cream freezer, numbers of viable cells decreased (Fig. 4). Apparent rate of death was greatest immediately after frozen product exited the freezer and slowed during storage. Thus, major freeze-damage occurred when probiotics were in the ice cream freezer. Probably damage to cells inside the ice cream freezer was caused by formation of ice crystals and by scraping of the cylinder wall by the blades of the freezer. Resistance to freezing damage differed between two probiotic strains.

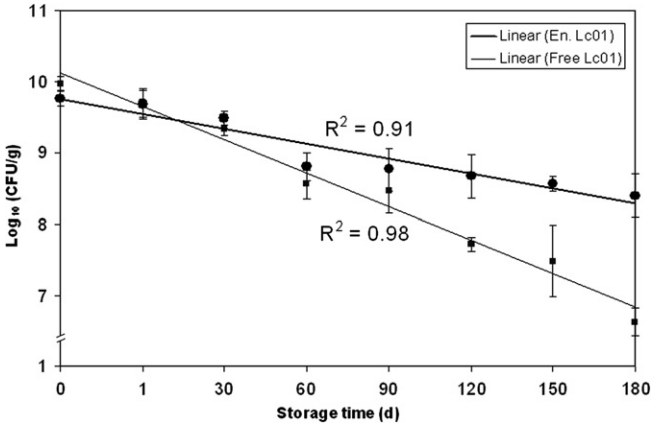

Fig. 2. Survival of free and encapsulated *L. casei* (*Lc-01*) cells in synbiotic ice cream during 180 days of storage at −20 °C. Error bars indicate standard error.

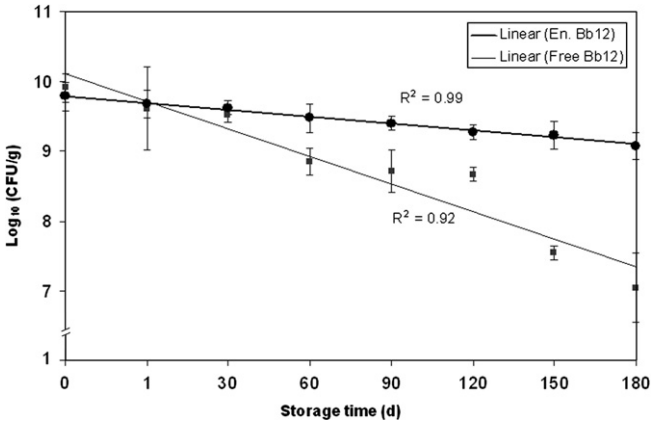

Fig. 3. Survival of free and encapsulated *B. lactis (Bb-12)* cells in synbiotic ice cream during 180 days of storage at −20 °C. Error bars indicate standard error.

Table 1
S-values of free and microencapsulated probiotic strains in synbiotic ice cream during 180 days storage at −20 °C

Storage days	Number of alive cells after expose to cold for different times (days) mean ± SE[a]			
	L. casei		*B. lactis*	
	Free	Encapsulated	Free	Encapsulated
0[b]	$(9.4 \pm 0.1) \times 10^9$	$(5.8 \pm 0.1) \times 10^9$	$(8.2 \pm 0.2) \times 10^9$	$(6.1 \pm 0.3) \times 10^9$
1	$(5.1 \pm 0.2) \times 10^9$	$(4.8 \pm 0.2) \times 10^9$	$(4.1 \pm 0.6) \times 10^9$	$(4.8 \pm 0.2) \times 10^9$
30	$(2.2 \pm 0.1) \times 10^9$	$(3.1 \pm 0.1) \times 10^9$	$(3.3 \pm 0.1) \times 10^9$	$(4.2 \pm 0.1) \times 10^9$
60	$(3.6 \pm 0.2) \times 10^8$	$(6.4 \pm 0.2) \times 10^8$	$(7.1 \pm 0.2) \times 10^8$	$(3.0 \pm 0.2) \times 10^9$
90	$(2.9 \pm 0.3) \times 10^8$	$(5.8 \pm 0.3) \times 10^8$	$(5.2 \pm 0.3) \times 10^8$	$(2.5 \pm 0.1) \times 10^9$
120	$(5.2 \pm 0.1) \times 10^7$	$(4.7 \pm 0.3) \times 10^8$	$(4.7 \pm 0.1) \times 10^8$	$(1.9 \pm 0.1) \times 10^9$
150	$(3.0 \pm 0.5) \times 10^7$	$(3.7 \pm 0.1) \times 10^8$	$(3.5 \pm 0.1) \times 10^7$	$(1.7 \pm 0.2) \times 10^9$
180	$(4.2 \pm 0.2) \times 10^6$	$(2.5 \pm 0.3) \times 10^8$	$(1.1 \pm 0.5) \times 10^7$	$(1.2 \pm 0.2) \times 10^9$
S_{30}-value[c] (days)	46.15 ± 0.8	111.11 ± 0.5	76.92 ± 1.2	85.71 ± 0.2
S_{150}-value[d] (days)	55.56 ± 0.4	137.62 ± 1.8	60.48 ± 0.1	277.78 ± 1.2
Determination coefficient (r^2)	0.98	0.91	0.92	0.99

[a] Mean of three replications ± standard error.
[b] Number of alive cells in ice cream mix before freezing.
[c] Survival value (S_{30}-value) is the time required to destroy one log cycle of the microorganism after 30 days.
[d] Survival value (S_{150}-value) is the time required to destroy 1 log cycle of the microorganism after 150 days.

Percentages of encapsulated cells found viable after 30 days frozen storage were about 54 and 69 for *L. casei* and *B. lactis*, respectively (Fig. 4). Percentages of survival among the free cells were much lower, about 22 and 40 for *L. casei* and *B. lactis*, respectively. Cells of *L. casei* were much larger (1.5−3 μm) than those of *B. lactis* (0.5−1.5 μm) (figures not shown) suggesting that the larger cells were much more susceptible to mechanical damage than smaller cells. Entrapped cells survived freezing better than free cells ($P < 0.05$) when compared within the same strain. Probiotics survived 30% more when they were encapsulated in calcium alginate than when they were not encapsulated. Protection by microencapsulation was significant ($P < 0.05$) both in the ice cream freezer and during frozen storage.

3.4. Sensory evaluations

The sensory scores of the synbiotic ice cream samples are given in Table 2. The points allocated for colour, body-texture and taste showed that the addition of free and encapsulated probiotics had no effect on sensory properties of non-fermented synbiotic ice cream. Yogurt or probiotic flavour was not found in all samples because of high pH of the non-fermented ice cream. Total evaluation in term of colour, texture and taste of all samples were good and did not have any marked off-flavour during the storage period. None of the ice creams were judged to be crumbly, weak, fluffy or sandy.

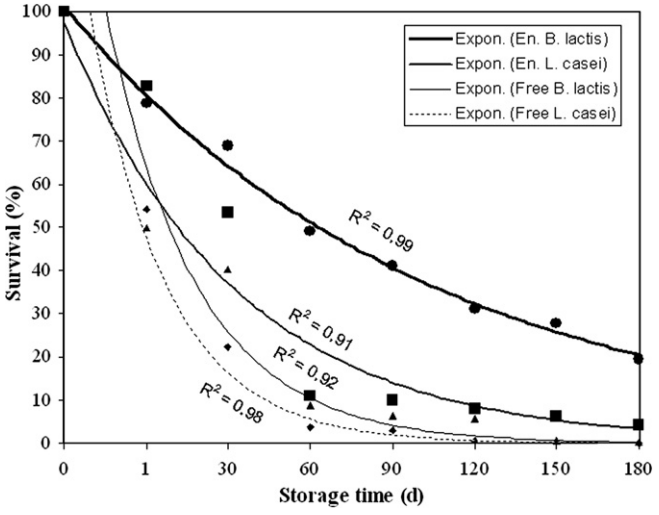

Fig. 4. Percent survival of free and encapsulated probiotic cells in synbiotic ice cream during 180 days of storage at −20 °C.

4. Conclusions

This study indicates that encapsulation can significantly improve the survival of probiotic bacteria in ice cream. Milk products such as ice cream and frozen desserts may serve as carriers for delivering the probiotic bacteria into the human gut. The high total solids level in ice cream including the fat and milk solids provides protection for the probiotic bacteria. There was a loss of only 0.7 and 0.4 log in the free state of *L. casei* and *B. lactis*, respectively, and 0.3 and 0.2 log in the encapsulated state over the first month and 2.7 and 2.5 log in the free state and 1.1 and 0.5 log in the encapsulated state over the subsequent five months, respectively. The final count after 6 months showed a 3.4 and 2.9 log decrease in the free state of *L. casei* and *B. lactis*, respectively, compared to 1.4 and 0.7 log in the encapsulated state. The numbers of viable

Table 2
Sensory properties of synbiotic ice cream

Samples	Color and appearance*	Body and texture	Flavor and taste	Total acceptability
	(1–5)	(1–5)	(1–10)	(1–20)
A	4.45 ± 0.07[a]	4.53 ± 0.02[ab]	9.15 ± 0.02[a]	18.13 ± 0.04[a]
B	4.40 ± 0.04[ab]	4.55 ± 0.01[a]	9.10 ± 0.05[ab]	18.05 ± 0.03[ab]
C	4.42 ± 0.03[ab]	4.56 ± 0.04[a]	9.13 ± 0.03[a]	18.11 ± 0.03[a]
D	4.43 ± 0.02[ab]	4.56 ± 0.05[a]	9.12 ± 0.04[a]	18.11 ± 0.04[a]
E	4.40 ± 0.03[ab]	4.54 ± 0.04[a]	9.14 ± 0.03[a]	18.08 ± 0.03[ab]

[a,b]Means in the same column followed by different letters were significantly different ($P < 0.05$).
A: Prebiotic ice cream with free *L. casei*, B: prebiotic ice cream with encapsulated *L. casei*, C: prebiotic ice cream with free *B. lactis*, D: prebiotic ice cream with encapsulated *B. lactis*, E: prebiotic ice cream without probiotic (control).
* Mean values from 32 panelists.

probiotic bacteria in all types of ice cream were between 10^8 and 10^9 cfu/g at the end of three months of storage which is the normal shelf life of ice cream. This viable cell number is higher than that recommended by the International Dairy Federation (10^7 cfu/g), indicating that the high initial number of probiotic can provide the recommended number in the final product. Further studies are needed to evaluate the protection effect of microencapsulation on the probiotic survival in the gastrointestinal tract.

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References

Akin, M. B., Akin, M. S., & 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 Chemistry, 104*, 93–99.

AOAC (1997). *Official methods of analysis* (16th ed., 3rd rev.). Arlington, VA: Association of Official Analytical Chemists.

Christiansen, P. S., Edelsten, D., Kristiansen, J. R., & Nielsen, E. W. (1996). Some properties of ice cream containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. *Milschwissenschaft, 51*, 502–504.

Dave, R. I., & Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. *Journal of Dairy Science, 81*, 2804–2816.

FAO/WHO (2001). Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. <http://www.fao.org/es/ESN/Probio/Probio.htm>.

Haynes, I. N., & Playne, M. J. (2002). Survival of probiotic cultures in low fat ice cream. *Australian Journal of Dairy Technology, 57*(1), 10–14.

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

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., & Yarmand, M. S. (2008a). Spectrophotometrically evaluation of probiotic growth in liquid media. *Asian Journal of Chemistry, 20*(3), 2414–2420.

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., & Yarmand, M. S. (2008b). Growth and survival of some probiotic strains in simulated ice cream conditions. *Journal of Applied Sciences, 8*(2), 379–382.

Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S., & Razavi, S. H. (2007b). Effect of lecithin and calcium chloride solution on the microencapsulation process yield of calcium alginate beads. *Iranian Polymer Journal, 16*(9), 597–606.

Homayouni, A., Ehsani, M. R., Mousavi, S. M., Valizadeh, M., & Djome, Z. E. (2005). Improving the quality of low-fat ice cream by hydrolyzing of casein micelles with chymosin (I) – Instrumental evaluation. *Iranian Journal of Agricultural Sciences, 36*(3), 765–773.

Homayouni, A., Ehsani, M. R., Mousavi, S. M., Valizadeh, M., & Djome, Z. E. (2006b). Improving the quality of low-fat ice cream by hydrolyzing of casein micelles with chymosin (II) – Sensory evaluation. *Iranian Journal of Agricultural Sciences, 36*(6), 1507–1515.

Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S., & Razavi, S. H. (2006a). A review on the method of increasing probiotic survival in functional dairy foods. In *Proceedings of the 9th Iranian nutrition congress* (pp. 288–297).

Homayouni, A., Ehsani, M. R., Azizi, A., Razavi, S. H., & Yarmand, M. S. (2007a). An introduction to functional dairy foods. In *Proceedings of the First National Functional Food Congress* (p. 60).

Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S. & Razavi, S. H. (2007c). Selection of appropriate probiotic strains for use in ice cream. In *Proceedings of the third IDF international symposium on ice cream* (p. 124).

ISIRI number 2450. (2005). Institute of Standards and Industrial Research of Iran. *Ice cream – Specification and test method (*1st. revision*).* Tehran, Iran.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp.. *Immunology and Cell Biology, 78*, 80–88.

Kailasapathy, K., & Sultana, k. (2003). Survival and β-D-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream. *Australian Journal of Dairy Technology, 58*(3), 223–227.

Kebary, K. M. K., Hussein, S. A., & Badawi, R. M. (1998). Improving viability of bifidobacterium and their effect on frozen ice milk. *Egyptian Journal of Dairy Science, 26*, 319–337.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2004). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *International Dairy Journal, 14*, 737–743.

Larisch, B. C., Poncelet, D., Champagne, C. P., & Neufeld, R. J. (1994). Microencapsulation of *Lactococcus lactis* subsp. cremoris. *Journal of Microencapsulation, 11*, 189–195.

Lourence, H. A., & Viljoen, B. (2002). Yogurt as probiotic carrier food. *International Dairy Journal, 11*, 1–17.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Selection and training of panel members. In *Sensory evaluation techniques* (3rd ed., pp. 146–172). New York: CRC Press.

Ravula, R. R., & Shah, N. P. (1998). Effect of acid casein hydrolyzates and cysteine on the viability of yogurt and probiotic bacteria in fermented frozen dairy desserts. *Australian Journal of Dairy Technology, 53*, 174–179.

Russ, J. C. (2005). *Image analysis of food microstructure*. Boca Raton, FL: CRC Press.

SAS (1996). *SAS user's guide: statistics, version 6.12 ed*. Gary, NC: SAS Institute.

Shah, N. P., & Ravula, R. R. (2000). Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. *Australian Journal of Dairy Technology, 55*, 139–144.

Sheu, T. Y., & Marshall, R. T. (1993). Microencapsulation of *Lactobacilli* in calcium alginate gels. *Journal of Food Science, 54*, 557–561.

Sheu, T. Y., Marshall, R. T., & Heymann, H. (1993). Improving survival of culture bacteria in frozen desserts by microentrapment. *Journal of Dairy Science, 76*, 1902–1907.

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. *International Journal of Food Microbiology, 62*, 47–55.

Vernazza, C. L., Rabiu, B. A., & Gibson, G. R. (2006). Human colonic microbiology and the role of dietary intervention: Introduction to prebiotics. In G. R. Gibson & R. A. Rastall (Eds.), *Prebiotics: Development & application* (pp. 1–12). England: John Wiley & Sons Ltd..

Wojtas, P. A., Hansen, L. T., & Paulson, A. T. (2008). Microstructural studies of probiotic bacteria-loaded alginate microcapsules using standard electron microscopy techniques and anhydrous fixation. *LWT-Food Science and Technology, 41*, 101–108.