

ENHANCEMENT OF PROBIOTIC VIABILITY IN ICE CREAM BY MICROENCAPSULATION

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Abstract: The aim of this study was to evaluate the survivability of two proven probiotic strains viz., *Lactobacillus acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) in ice cream using microencapsulation technique. Four types of probiotic ice cream containing free and microencapsulated *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298), were manufactured. The survival of *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) were monitored during the storage period of 180 days at -23°C . The viable cell count of *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) in the free state in prepared ice cream mixture was $5.1 \pm 0.2 \times 10^9$ cfu/ ml and $4.3 \pm 0.2 \times 10^9$ cfu/ ml at day one and the numbers were decreased to $4.1 \pm 0.3 \times 10^6$ and $1.9 \pm 0.2 \times 10^7$ cfu/ ml after 180 days of storage at -23°C respectively. After encapsulation of the two probiotic bacteria along with calcium alginate and whey protein concentrate beads, the probiotic survival rate raised at of 30 per cent during the same period of storage. The present study envisaged that microencapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended period of shelf-life. Further the addition of microencapsulated probiotics in ice cream had no significant effect on the sensory properties.

Keywords: Ice cream, Microencapsulation, Probiotic bacteria, Sodium alginate, Survival.

Introduction

Ice cream is a delicious, wholesome, nutritious frozen dairy product, which is widely consumed in different parts of the world and it is very popular among all sections of the people because of the taste delight to nutrient delivery. Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO, 2001).

Awareness among the consumers on diet related health issues and evidence regarding acquiring health benefits of probiotics have increased the consumer's demand for probiotic foods all over the world. Among the most used organisms are those belonging to the genera of *Lactobacillus* and *Bifidobacterium*, which are believed to have beneficial effects on human health (Saxelin *et al.*, 2005). Development of probiotic dairy products is a key research

priority for food design and a challenge for both industry and science sectors. Some of the reported nutritional and physiological benefits of probiotic foods are promotion of growth and digestion, setting effect on the gastro intestinal tract, improving bowel movement, suppression of cancer, catering to lactose intolerance and lowering blood cholesterol level etc.

The therapeutic value of any probiotic food normally depends on the viability of these bacteria. 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. (Hekmat and McMahan, 1992; Kailasapathy and Sultana, 2003). Encapsulation helps to isolate the bacterial cells from the effects of the hostile environment and enhance their viability during processing and also for their targeted delivery in gastrointestinal tract, thus potentially preventing cell loss (Kearney *et al.*, 1990; Shah and Ravula, 2000).

The objective of this study was to evaluate the survival of microencapsulated and free probiotic culture in ice cream over a period of 180 days storage at $-23\text{ }^{\circ}\text{C}$ by using sodium alginate and whey protein concentrate beads.

Materials and Methods

Method of encapsulation of probiotics

Glass wares and solutions used in the protocols were sterilized at $121\text{ }^{\circ}\text{C}$ for 15 min. Alginate beads were produced using a modified encapsulation method (King., 1995; Sultana *et al.*, 2000; Krasaekoopt *et al.*, 2003). A probiotic cell suspension was prepared by centrifuging 80 ml of 24 hour old culture at 5000X G for 15 minutes. The cells were washed twice with saline solution (20 ml). The wall materials were sodium alginate (2.0%w/v) + starch (0.5%w/v) and sodium alginate (2.0%w/v) + whey protein concentrate (1.0%w/v) + starch (0.5w/v). To form capsules, a cell suspension was mixed with a 60 ml of wall material solution and the mixture was dripped into a solution containing CaCl_2 as the divalent cation. The CaCl_2 concentration was at 0.1M and dripping was achieved with a sterile syringe with different size of needles (21G, 26G and insulin syringe). The distance between syringe and CaCl_2 solution was 30 cm. The droplets formed gel spheres instantaneously, entrapping the cells in a three dimensional lattice of ionically cross linked alginate.

Ice cream making procedure:

Ice cream mix was prepared to contain a final composition of 10 per cent fat, 36 per cent total solids, 15 per cent sugar, 0.5 per cent stabilizer and emulsifier in the ice cream, the mix ingredients were homogenized as described by Arbuckle. (1986) and then heated to 80°C for

30 sec. Mixes were cooled to 5°C and aged for 4 hrs. After ageing the ice cream mix was heat treated to a temperature of 80°C for 30 sec and cooled to 40°C. Two probiotic strains viz., *Lactobacillus acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) were inoculated into ice cream mix at the rate of 4 per cent level and incubated at 40°C until the pH of 5.5 is reached (Hekmat and McMahon, 1992). The culture could reach the pH of 5.5 within 4 hours and the probiotic count of 1×10^6 cfu has been reached within 4 hours. Then the ice cream mix was frozen at -4 to -5°C and stored at -23°C where the ice cream was hardened.

Enumeration of free and encapsulated probiotics

Enumeration of probiotic bacteria was achieved as described by Haynes and Playne (2002). All enumerating plates of *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) were incubated at 37°C for 72 hour under aerobic and anaerobic conditions, respectively. The averages of all results were expressed as colony-forming units per gram of sample (CFU g⁻¹). The entrapped bacteria were released from the beads was counted in ice cream as per the procedure described by Sheu and Marshall (1993).

Analysis of beads

The beads prepared from extrusion method were stored in 0.1M CaCl₂ solution and water at 37°C for one day and observed under light microscope for their size and shape. The size was measured by using stage micrometer, 100 beads were measured for each sample and the average bead size was recorded before and after storage. The calcium alginate beads were stained with safranin and its diameter was measured at 10X. At least 100 randomly selected beads were measured for each sample.

Physico-chemical analysis

The pH of the ice cream was measured using a digital pH-meter (H1 2211 Ph/ORP Meter, Hanna Instruments). The fat contents of milk and ice cream were determined using the Gerber method. All chemical measurements were done in triplicate.

Sensory analysis

Microencapsulated probiotic ice cream samples were organoleptically analysed by 24 panelists using a sensory rating scale of 1–10 for flavor and taste, 1–5 for body and texture and 1–5 for colour and appearance, as described by Homayouni *et al.* (2006b).

Statistical analysis

The data collected on various parameters were subjected to analysis of variance (ANOVA) procedure. The data were analyzed by approved statistical methods of SPSS (Statistical Package for the Social Sciences).

Results

Chemical and physical characteristics

The chemical composition of the cow milk used in the production of probiotic ice cream was: pH 6.60 ± 0.02 , titratable acidity 6.58 ± 0.02 and fat $3.90 \pm 0.02\%$. The dry matter and fat content of the ice cream mixture was: $39.31 \pm 0.12\%$ and $9.04 \pm 0.03\%$, respectively. The overrun value was 95 ± 2.0 . The respective mean value of fresh extrusion beads in CaCl_2 and water were 3.0 ± 0.14 , $3.0 \pm 0.12\text{mm}$ and 24 hrs stored beads were $2.6 \pm 0.10\text{mm}$, $2.9 \pm 0.11\text{mm}$ respectively.

Survival of free and encapsulated bacteria in ice cream

Viability of *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) were enumerated at day one and at the end of every 30 days until 180 days of storage. The viable counts were showed in table-1 and table-2. Unencapsulated free *L. acidophilus* (LA-5), the cell number dropped substantially from $5.1 \pm 0.2 \times 10^9$ to $4.1 \pm 0.3 \times 10^6$ (about 3 log number) from day one to 180 days of storage at -23°C , wherein microencapsulated *L. acidophilus* (LA-5), the cell number decreased from $4.4 \pm 0.3 \times 10^9$ to $2.3 \pm 0.2 \times 10^8$ (about a log number). The *Lactobacillus casei* (NCDC-298) count showed an average 3 log reduction in free state from $4.3 \pm 0.2 \times 10^9$ to $1.9 \pm 0.2 \times 10^7$ during day one to 180 days, wherein microencapsulated state of the same strains showed a decreased count from $4.7 \pm 0.4 \times 10^9$ to $1.5 \pm 0.7 \times 10^9$ respectively.

The probiotic survivability was expressed as the survival value (S-value), this defined as the time required destroying 90% or one log cycle of the organism. The S-values of both free cells and microencapsulated probiotics in ice cream during 180 days storage at -23°C are shown in Table 1 and 2. The S-values of unencapsulated free and microencapsulated *L. acidophilus* (LA-5) at 30 days were 41.12 ± 0.7 and 109.09 ± 0.7 respectively. Whereas The S-values of unencapsulated free and microencapsulated *Lactobacillus casei* (NCDC-298) at 180 days were 63.46 ± 0.5 and 239.51 ± 1.9 respectively.

Sensory analysis

Sensory analysis of probiotic ice cream were showed in the table-3. The overall acceptability in terms of colour, texture and taste of free and microencapsulated *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) samples were 17.96 ± 0.03 , 17.92 ± 0.04 , 17.99 ± 0.02 and 18.06 ± 0.03 respectively.

Discussion

Chemical and physical characteristics

There is no significant difference in the bead size of extrusion method with two different wall materials, but increase in size with increasing size of needle was observed in this study, which is similar with the findings of Ozer *et al.*(2008) he revealed that the bead size ranged from 0.5-1.0 mm diameter when 0.6 mm syringe are used for dripping in extrusion method and bead size 3-4 mm diameter was observed when using 21G needle. Sheu *et al.* (1993) reported that large beads might cause coarseness of texture in ice milk and ice cream and very small beads did not provide sufficient protection of the probiotic bacteria.

Survival of free and encapsulated bacteria in ice cream

The survivability of *L. acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) were recorded between the free and encapsulated states in probiotic ice cream at the end of 180 days frozen storage showed a significant difference ($P < 0.05$). The present results are in accordance with Shah and Ravula (2000). who 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 and similarly, In frozen ice milk, 40% more lactobacilli survived when they were entrapped in calcium alginate beads (Sheu and Marshall, 1993).

Comparison of S-value after 30 and 180 days of storage revealed that freezing process had significant ($P < 0.05$) effect on the viability of free cells. Further, microencapsulated cells required longer time to decrease one log cycle in viable counts. Therefore, microencapsulation of probiotic bacteria in beads with diameter between 2-3mm can increase the viability of probiotics.

From this study, the numbers of viable probiotic bacterial cells decreased, when they were added to the ice cream mixture and then frozen in ice cream freezer. Probiotic bacterial cell death was greatest immediately after frozen product exited the freezer and slowed during storage. The major freeze-damage occurred when probiotics were in the ice cream freezer. further damage to cells inside the ice cream freezer was probably due to formation of ice crystals and by scraping of the cylinder wall by the blades of the ice cream freezer.

Further, it has been found that the resistance to freezing damage was differed between two probiotic strains. The percent average of encapsulated cells found viable after 30 days frozen storage were about 53 and 69 per cent for *L.acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) respectively and survival among the free cells were much lower, about 25 and

44 per cent for *L.acidophilus* (LA-5) and *Lactobacillus casei* (NCDC-298) respectively. Microencapsulated cells survived freezing better than free cells ($P < 0.05$) when compared within the same strain. 30 per cent more survival rate was observed when the probiotics were encapsulated in calcium alginate than when they were not encapsulated. Protection by microencapsulation was significant ($P < 0.05$) in the ice cream freezer as well as during frozen storage. These results were in close agreement with findings of Homayouni *et al.* (2008).

Sensory analysis

The scores of sensory analysis of the probiotic ice cream samples for colour, body-texture and taste showed that the addition of free and encapsulated probiotics in ice cream had no effect on sensory properties of probiotic ice cream (Table 3). Overall acceptability in terms of colour, texture and taste of all samples were good and no marked off-flavour was found during the storage period.

Conclusions

The study indicates that probiotic survivability in ice cream can significantly improved by microencapsulation. High fat and solids content of ice cream and other frozen desserts may provide protection to the probiotic bacteria and serve as carrier for delivering the probiotic bacteria into the human gut. In all types of ice cream the number of viable probiotic bacterial count 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 of the International Dairy Federation recommendations (10^7 cfu/g), As the efficient delivery of live cultures represents a major challenge in probiotic product development, the results of the present study demonstrated that the potential of increasing both the technological suitability and expanding the performance of probiotic strains can be done through encapsulation techniques. It is concluded that the incorporation of encapsulated probiotic strains in dairy products can result in more efficacious and diverse probiotic products in the future, leading ultimately to improved consumer health.

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Table 1. Viability of free and microencapsulated *L. acidophilus* (LA-5) strain in probiotic ice cream during different the storage

Storage (in days)	Free <i>L. acidophilus</i> (LA-5) in cfu/ ml	Microencapsulated <i>L. acidophilus</i> (LA-5) in cfu/ ml
0 ^b	$(8.2 \pm 0.2) \times 10^9$	$(5.5 \pm 0.2) \times 10^9$
1	$(5.1 \pm 0.2) \times 10^9$	$(4.4 \pm 0.3) \times 10^9$
30	$(2.1 \pm 0.3) \times 10^9$	$(3.0 \pm 0.5) \times 10^9$
60	$(3.3 \pm 0.2) \times 10^8$	$(6.2 \pm 0.4) \times 10^8$
90	$(2.3 \pm 0.1) \times 10^8$	$(5.5 \pm 0.2) \times 10^8$
120	$(5.2 \pm 0.2) \times 10^7$	$(4.6 \pm 0.2) \times 10^8$
150	$(3.3 \pm 0.2) \times 10^7$	$(3.3 \pm 0.1) \times 10^8$
180	$(4.1 \pm 0.3) \times 10^6$	$(2.3 \pm 0.2) \times 10^8$
S ₃₀ -value ^c (days)	41.12 ± 0.7	109.09 ± 0.7
S ₁₈₀ -value ^c (days)	52.61 ± 0.8	140.11 ± 1.8

^a Mean of three replications ± standard error.

^b Number of alive cells in ice cream mix before freezing.

^c Survival value (S₃₀ & S₁₈₀-value) is the time required to destroy one log cycle of the microorganism after 30 days and 180 days.

Table 2. Viability of free and microencapsulated *Lactobacillus casei* (NCDC-298) strain in probiotic ice cream during different the storage.

Storage (in days)	Free <i>Lactobacillus casei</i> (NCDC-298) in cfu/ ml	Microencapsulated <i>Lactobacillus casei</i> (NCDC-298) in cfu/ ml
0 ^b	$(8.2 \pm 0.1) \times 10^9$	$(6.5 \pm 0.4) \times 10^9$
1	$(4.3 \pm 0.2) \times 10^9$	$(4.7 \pm 0.4) \times 10^9$
30	$(3.5 \pm 0.3) \times 10^9$	$(4.4 \pm 0.2) \times 10^9$
60	$(6.3 \pm 0.3) \times 10^8$	$(3.7 \pm 0.2) \times 10^9$
90	$(5.3 \pm 0.1) \times 10^8$	$(2.4 \pm 0.3) \times 10^9$
120	$(4.2 \pm 0.2) \times 10^8$	$(1.6 \pm 0.6) \times 10^9$
150	$(3.4 \pm 0.1) \times 10^7$	$(1.4 \pm 0.8) \times 10^9$
180	$(1.9 \pm 0.2) \times 10^7$	$(1.5 \pm 0.7) \times 10^9$
S ₃₀ -value ^c (days)	81.63 ± 1.3	89.51 ± 0.7
S ₁₈₀ -value ^c (days)	63.46 ± 0.5	239.51 ± 1.9

^a Mean of three replications ± standard error.

^b Number of alive cells in ice cream mix before freezing.

^c Survival value (S₃₀ & S₁₈₀-value) is the time required to destroy one log cycle of the microorganism after 30 days and 180 days.

Table 3. Sensory properties of probiotic ice cream

Icecream Samples contains	Colour and appearance(1-5)	Flavour and taste(1-5)	Body and texture(1-10)	Overall acceptability
Free <i>L. acidophilus</i> (LA-5)	4.33 ± 0.05 ^a	4.42 ± 0.04 ^{ab}	9.21 ± 0.02 ^a	17.96 ± 0.03 ^a
Microencapsulated <i>L. acidophilus</i> (LA-5)	4.32 ± 0.04 ^{ab}	4.49 ± 0.01 ^a	9.11 ± 0.05 ^{ab}	17.92 ± 0.04 ^{ab}
Free <i>Lactobacillus casei</i> (NCDC-298)	4.37 ± 0.02 ^{ab}	4.45 ± 0.03 ^a	9.17 ± 0.03 ^a	17.99 ± 0.02 ^a
Microencapsulated <i>Lactobacillus casei</i> (NCDC-298)	4.40 ± 0.03 ^{ab}	4.47 ± 0.04 ^a	9.19 ± 0.04 ^a	18.06 ± 0.03 ^a
Without probiotics	4.31 ± 0.04 ^{ab}	4.62 ± 0.03 ^a	9.17 ± 0.03 ^a	18.10 ± 0.03 ^{ab}

Means in the same column followed by different superscripts were significantly different (P < 0.05).