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Augmentation of Probiotic Viability in Ice Cream Using Microencapsulation Technique

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Abstract This study was aimed to evaluate the survivability of two proven probiotic strains viz., *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis* (BB-12*)* in ice cream using microencapsulation technique. Four different types of probiotic ice cream viz., free and encapsulated *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis* (BB-12*)* were manufactured. Probiotic viability of these strains was monitored during different storage period upto 180 days at -23°C. The viable cell count of *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* in the free state in prepared ice cream mixture was $5.3 \pm 0.2 \times 10^9$ cfu/ ml and $4.6 \pm 0.2 \times 10^9$ cfu/ ml at day one and the numbers were decreased to $4.5 \pm 0.2 \times 10^6$ and $2.1 \pm 0.1 \times 10^7$ cfu/ ml in ice-cream after 180 days of storage at -23°C respectively. After the procedure of microencapsulation of *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* along with calcium alginate and whey protein concentrate beads, the probiotic survivability raised at the rate of above 30 percent during the same period of storage. The present study revealed 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 *Probiotic Survival; Sodium Alginate; Whey Protein Concentrate*

1. 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). Probiotics have been recently defined as “live microbes which transit the gastro-intestinal tract and in doing so benefit the health of the consumer (Tannock *et al.,* 2000)

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. The addition of probiotic micro-organisms to various foods in order to enhance their nutritive value and potential health benefits is currently of great interest.

The genera of *Lactobacillus* and *Bifidobacterium* are most used probiotic micro-organisms which are believed to have beneficial effects on human health (Saxelin *et al.,* 2005). Owing to the perceived health benefits, probiotics have been incorporated into a range of dairy products including ice cream, yoghurt, cheese, milk powder and frozen dairy desserts.

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. Some authors have shown that the freezing process affects dramatically the number of live probiotic cells (Hekmat and McMahon, 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. Microencapsulation protects probiotic organisms during freezing, freeze drying and also improves the survival of probiotic bacteria in frozen desserts (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°C by using sodium alginate and whey protein concentrate beads.

2. Materials and Methods

2.1. Activation of Probiotic Cultures

Freeze-dried pure probiotic culture of *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* were obtained from NCDC (Karnal) and CHR-Hansen (Horsholm, Denmark) were activated by inoculating in the MRS-broth at 37°C for 24 hour. The probiotic biomass in late-log phase was harvested by centrifugation at 5000X G for 10 min at 4°C and then washed twice in sterile 0.9 per cent saline under the same centrifugation conditions and used in the microencapsulation process.

2.2. Method of Encapsulation of Probiotics

All glass wares and solutions used in the protocols were sterilized at 121°C for 15 min. Alginate beads were produced using a modified encapsulation method (Sultana *et al*., 2000; Krasaekoopt *et al*., 2003; Kebary *et al*., 1998). 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.

2.3. Procedure for Making of Probiotic Ice Cream

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 (Rothwell, 1976; 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 casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* in free and encapsulated form 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. Then the ice cream mix was freezed at – 4 to – 5°C and stored at – 23°C where the ice cream was hardened.

2.4. Enumeration of Free and Encapsulated Probiotics

Enumeration of probiotic bacteria was achieved as described by (Haynes and Playne, 2002; Lourence, 2002). Probiotic bacterial counts were enumerated before and immediately after freezing as well as at the end of every 30 days until 180 days of storage at ⁻23°C. The samples (10 g) of ice cream mixture prior and after freezing 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.

All enumerating plates of *L. acidophilus (LA-5)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* 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).

2.5. Analysis of Beads

The beads prepared from extrusion method were stored in 0.1M $CaCl_2$ solution and water at 37^0C 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.

2.6. Analysis of Physico-Chemical Properties

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. The overrun of the final product was determined using the following formula (Homayouni *et al.,* 2005).

$$\text{Overrun} = \frac{\text{Weight of Unit Mix} - \text{Weight of Equal Volume of Ice Cream}}{\text{Weight of Equal Volume of Ice Cream}}$$

2.7. Analysis of Sensory Properties

Sensory properties of 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).

2.8. 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).

3. Results

3.1. Chemical and Physical Characteristics

The chemical composition of the cow milk used in the production of probiotic ice cream was: pH 6.57 ± 0.01, titratable acidity 0.22 ± 0.04 and fat 3.90 ± 0.02%. The dry matter and fat content of the ice cream mixture was: 39.31 ± 0.12% and 9.04± 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±0.12mm and 24 hrs stored beads were 2.6±0.10mm, 2.9±0.11mm respectively.

3.2. Survivability of Free and Encapsulated Bacteria in Ice Cream

Survivability of two proven bacteria viz., *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* 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 *Lactobacillus casei (NCDC-298)*, the cell number dropped substantially from $5.3 \pm 0.2 \times 10^9$ to $4.5 \pm 0.2 \times 10^6$ (about 3 log number) from day one to 180 days of storage at −23°C, wherein microencapsulated *Lactobacillus casei (NCDC-298)*, the cell number decreased from $4.8 \pm 0.2 \times 10^9$ to $2.6 \pm 0.3 \times 10^8$ (about a log number). The *Bifidobacterium animalis ssp. Lactis (BB-12)* count showed an average 2 log reduction in free state from $4.6 \pm 0.2 \times 10^9$ to $2.1 \pm 0.1 \times 10^7$ during day one to 180 days, wherein microencapsulated state of the same strains showed a decreased count from $4.5 \pm 0.3 \times 10^9$ to $1.8 \pm 0.6 \times 10^9$ respectively.

Table 1: *Viability of Free and Microencapsulated Lactobacillus Casei (NCDC-298) Strain in Probiotic Ice Cream during Different Storage*

Storage (in days)	Free *Lactobacillus Casei (NCDC-298)* in cfu/ ml	Microencapsulated *Lactobacillus Casei (NCDC-298)* in cfu/ ml
0[b]	$(8.7 \pm 0.1) \times 10^9$	$(5.8 \pm 0.2) \times 10^9$
1	$(5.3 \pm 0.2) \times 10^9$	$(4.8 \pm 0.2) \times 10^9$
30	$(2.4 \pm 0.1) \times 10^9$	$(3.2 \pm 0.4) \times 10^9$
60	$(3.5 \pm 0.1) \times 10^8$	$(6.6 \pm 0.3) \times 10^8$
90	$(2.4 \pm 0.2) \times 10^8$	$(5.4 \pm 0.3) \times 10^8$
120	$(5.6 \pm 0.4) \times 10^7$	$(4.8 \pm 0.1) \times 10^8$
150	$(3.4 \pm 0.2) \times 10^7$	$(3.2 \pm 0.1) \times 10^8$
180	$(4.5 \pm 0.2) \times 10^6$	$(2.6 \pm 0.3) \times 10^8$
S_{30}-value[c] (days)	40.13 ± 0.7	106.06 ± 0.7
S_{180}-value[c] (days)	56.61± 0.8	150.11± 1.8

[a] Mean of three replications ± standard error

[b] Number of alive cells in ice cream mix before freezing

[c] Survival value (S_{30}&S_{180}-value) is the time required to destroy one log cycle of the microorganism after 30 days and 180 days

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 *Lactobacillus casei (NCDC-298)* at 30 days were 40.13 ± 0.7 and 106.06 ± 0.7 respectively. Whereas The S-values of unencapsulated free and microencapsulated *Bifidobacterium animalis ssp. Lactis (BB-12)* at 180 days were 61.43 ± 0.6 and 207.42 ± 1.7 respectively.

Table 2: *Viability of Free and Microencapsulated Bifidobacterium Animalis Ssp. Lactis (BB-12) Strain in Probiotic Ice Cream during Different Storage*

Storage (in days)	Free *Bifidobacterium Animalis Ssp. Lactis (BB-12)* in cfu/ ml	Microencapsulated *Bifidobacterium Animalis Ssp. Lactis (BB-12)* in cfu/ ml
0[b]	$(8.6 \pm 0.2)\times10^{9}$	$(6.7 \pm 0.3) \times10^{9}$
1	$(4.6 \pm 0.2) \times 10^{9}$	$(4.5 \pm 0.3) \times10^{9}$
30	$(3.8 \pm 0.1) \times10^{9}$	$(4.3 \pm 0.1) \times10^{9}$
60	$(6.5 \pm 0.4) \times 10^{8}$	$(3.9 \pm 0.3) \times 10^{9}$
90	$(5.7 \pm 0.2) \times 10^{8}$	$(2.6 \pm 0.7) \times10^{9}$
120	$(5.0 \pm 0.1) \times 10^{8}$	$(1.8 \pm 0.5) \times 10^{9}$
150	$(3.6 \pm 0.2) \times 10^{7}$	$(1.9\pm 0.6) \times10^{9}$
180	$(2.1 \pm 0.1) \times 10^{7}$	$(1.8 \pm 0.6) \times10^{9}$
S_{30}-value[c] (days)	82.43 ± 1.4	91.53 ± 0.6
S_{180}-value[c] (days)	61.43± 0.6	207.42± 1.7

[a] Mean of three replications ± standard error

[b] Number of alive cells in ice cream mix before freezing

[c] Survival value (S_{30}&S_{180}-value) is the time required to destroy one log cycle of the microorganism after 30 days and 180 days

3.3. Sensory Analysis

Sensory analysis of probiotic icecream was showed in the Table 3. The overall acceptability in terms of colour, texture and taste of free and microencapsulated *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* samples were 17.83 ± 0.09, 18.03 ± 0.11, 18.08 ± 0.09 and 18.03 ± 0.18 respectively.

Table 3: *Sensory Properties of Probiotic Ice Cream*

Ice-cream Samples Contains	Colour and Appearance (1-5)	Flavors and Taste (1-5)	Body and Texture (1-10)	Overall Acceptability
Free *L. acidophilus (LA-5)*	4.21 ± 0.04[a]	4.30 ± 0.03[ab]	9.32± 0.02[a]	17.83 ± 0.09[a]
Microencapsulated *L. acidophilus (LA-5)*	4.40 ± 0.03[ab]	4.40 ± 0.02[a]	9.23 ± 0.06[ab]	18.03 ± 0.11[ab]
Free *Bifidobacterium animalis ssp. Lactis (BB-12)*	4.41 ± 0.03[ab]	4.44 ± 0.02[a]	9.23 ± 0.04[a]	18.08 ± 0.09[a]
Microencapsulated *Bifidobacterium animalis ssp. Lactis (BB-12)*	4.39 ± 0.08[ab]	4.42 ± 0.05[a]	9.22 ± 0.05[a]	18.03 ± 0.18[a]
Without probiotics	4.30 ± 0.03[ab]	4.63 ± 0.02[a]	9.20± 0.02[a]	18.13 ± 0.07[ab]

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

4. Discussion

4.1. 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.

4.2. Survival of Free and Encapsulated Bacteria in Ice Cream

The survivability of *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* 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 *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* respectively and survival among the free cells were much lower, about 25 and 44 per cent for *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis ssp. Lactis (BB-12)* 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).

4.3. 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-flavor was found during the storage period.

5. 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. In addition, dairy foods provide an ideal food delivery system of probiotic bacteria to the human gut to promote growth or support viability of these cultures. 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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