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Research Article

EVALUATION OF VIABILITY OF CO-ENCAPSULATED PRE- AND CERTAIN PROBIOTICS IN ICE CREAM DURING FROZEN STORAGE

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ABSTRACT
A study was conducted to investigate the effect of supplementation of co-encapsulated probiotic organisms (*Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231) along with prebiotics (3% FOS) using 2% sodium alginate as encapsulating material in ice cream on viability of probiotics, certain physicochemical properties and sensory evaluation of ice cream on initial, 15th, 30th, 45th, 60th, 75th and 90th day of frozen storage. Five categories of ice cream mixes of 2.5 Kg each were formulated as per standard method. Control ice cream was made without supplementing probiotics in the ice cream mix, treatment I was supplemented with encapsulated *Lactobacillus helveticus* 194 (@ 8.77 log $_{10}$ cfu/g), treatment II was supplemented with non encapsulated *Lactobacillus helveticus* 194 (@ 8.60 log $_{10}$ cfu/g), treatment III was supplemented with encapsulated *Bifidobacterium bifidum* 231 (@ 8.90 log $_{10}$ cfu/g) and treatment IV was supplemented with non encapsulated *Bifidobacterium bifidum* 231 (@ 8.85 log $_{10}$ cfu/g). The prepared ice cream samples were packaged in polystyrene cups aseptically and stored at -20^{0}C. The results showed that the mean pH values of probiotic ice cream decreased from initial day to 90 days, whereas the mean titra table acidity values increased from initial day to 90 days of frozen storage. The viable counts of non encapsulated *Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231 in ice cream were 8.16 and 8.23 $\log_{10}$cfu/g on initial day and decreased to 6.06 and 6.33 $\log_{10}$cfu/g during 90 days of frozen storage, whereas encapsulated probiotic bacterial cell counts were 7.96 and 8.06 log $_{10}$ cfu/g, respectively at the end of storage period. The addition of probiotic cultures either in encapsulated and non encapsulated states did not significantly affect the colour and appearance, flavour, body and texture and overall acceptability of ice cream over a storage period of 90 days at -20^{o}C. The microencapsulation with prebiotics appears to enhance the survival abilities of probiotic bacteria in ice cream during freezing and frozen storage.

Key Words: *Co-Encapsulation, Prebiotics, Probiotics, Viability, Ice Cream*

INTRODUCTION
Nowadays, consumers are increasingly interested in their personal health and expect the food they eat to be healthy or even capable of preventing illness (Mattila-Sandholm *et al.,* 2002). Prebiotics are 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon and thus improve host health (Gibson, 2004). Probiotics have been defined as ''live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'' (FAO/WHO, 2001). Most of the probiotic organisms belong to the genera of *Lactobacillus* and *Bifidobacterium*, which are believed to have beneficial effects on human health (Saxelin *et al.,* 2005). These benefits include improvement to the intestinal microbial balance, reduction of blood cholesterol, prevention of inflammatory bowel disease, reduction of the risk associated with mutagenicity and carcinogenicity, stimulation of the immune system and reduction in the incidence of constipation, diarrhoea and lactose intolerance (O'May and MacFarlane, 2005 and Cogan *et al.,* 2007). The efficiency of added probiotic bacteria depends on the dose level, storage temperature, type of dairy foods and presence of air (Homayouni *et al.,* 2006), their viability must be maintained throughout the

product's shelf-life (Kailasapathy and Chin, 2000). Hence viability of probiotic bacteria is of paramount importance in the marketability of probiotic-based food products. 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 (Kailasapathy and Sultana, 2003 and Akin and Kirmaci, 2007). Some authors have shown that the freezing process affects dramatically the number of live probiotic cells (Kailasapathy and Sultana, 2003). The co-encapsulation of probiotic with prebiotic improved the survival rate of probiotics (Chen *et al.,* 2005). Encapsulation has been investigated for improving the viability of microorganisms in both dairy products and the GI tract (Krasaekoopt *et al.,* 2003 and Picot and Lacroix, 2004). In the present study an attempt was made to assess the effect of supplementation of co-encapsulated pre and certain probiotics on the viability of probiotics and on physic -chemical and sensory properties of ice cream during freezing and frozen storage.

MATERIALS AND METHODS

Probiotic Cultures

The probiotic cultures namely, *Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231 were procured from National Dairy Research Institute, Karnal, Haryana. The lyophilized cultures were reconstituted into MRS broth and incubated at 37^0C for 48 h. The cultures were then sub-cultured in MRS broth and incubated at 37^0C for 48 h to get pure colonies. It was then properly activated and served as the inoculum. Probiotics were grown in MRS broth for production of freeze dried *Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231 using 5% inoculum seperately and incubated for 48 h at 37^0C and then the cells were harvested by centrifugation at 5000 rpm for 15 minutes at 4^0C and washed with 0.9% normal saline and lyophilised to get powder form of culture and stored at 4^0C. Probiotic culture counts were determined by plating on MRS agar (Merck) and incubating anaerobically at 37 ± 1°C for 72 h as per the procedure described by Christiansen *et al.,* (1996).

Prebiotic Sugars

Commercial FOS-P (fructooligosaccharides-p powder) containing 2.1% w/w moisture, Carbohydrate composition (% dry basis) of 3.8 and Glucose + Fructose + Sucrose and Fructooligosaccharides-96.2 was procured from Xena BioHerbals, Hyderabad.

Microencapsulation of Probiotic Cultures

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 3% FOS (Merck, Germany) and 10^8 CFU per gram probiotic cultures. The alginate mixture was stirred vigorously until a homogenous solution is formed. The alginate mixture is atomised by compressed N_2 gas pressure through micro encapsulator such that the formed alginate beads drops into 0.1M $CaCl_2$ for hardening of capsules. Freshly formed capsules were rinsed twice with distilled water and dried. The microcapsules were dried in hot air oven at 45°C for 48h.

Manufacture of Probiotic Ice Cream

Probiotic ice cream was manufactured according to Criscio *et al.,* (2010). Five categories of ice cream mixes of 2.5 Kg each were formulated. All mixes were standardised to contain 10% fat, 11% solids non fat, 16% sugar and 0.3% stabiliser (sodium alginate). The ice cream mixes were heated to 80°C for 30 min and then cooled and aged at 5°C for 4 h before freezing. The 12.5 kg of ice cream mix was divided into 5 batches and the freeze dried probiotic cultures were added.

Treatments

Ice cream manufactured without any probiotics was kept as a control, treatment I was supplemented with encapsulated *Lactobacillus helveticus* 194 (@ 8.77 $\log_{10}$ cfu/g), treatment II was supplemented with non encapsulated *Lactobacillus helveticus* 194 (@ 8.60 $\log_{10}$ cfu/g), treatment III was supplemented with encapsulated *Bifidobacterium bifidum* 231 (@ 8.90 $\log_{10}$ cfu/g) and treatment IV was supplemented with

non encapsulated *Bifidobacterium bifidum* 231 (@ 8.85 cfu/g). The ice cream samples were evaluated for physical, bacteriological and organoleptic properties on initial, 15, 30, 45, 60, 75 and 90 days of frozen storage.

Analysis of Probiotic Ice Cream

Determination of Titratable Acidity and pH

Titratable acidity of ice cream samples was determined according to IS: 1479 (Part-I) 1960. The pH of the ice cream samples was measured using pH meter (Hanna make).

Bacteriological Analysis

The ice cream samples were examined to enumerate viability of probiotic organisms at an interval of 15 days for a period of 90 days. MRS Agar medium for enumeration of the probiotic organisms. Selective enumeration of *Lactobacillus helveticus* was done by adding rifampicin (100µg/ml) to the MRS Agar (Jayalalitha *et al.,* 2011).

Enumeration of Encapsulated Probiotic Bacteria

One gram of product was suspended in 9 ml of sterile 0.1M phosphate buffer (pH 7.0) and the contents were mixed at high speed in a vortex mixer for 15 minutes, serially diluted using sterile normal saline and plated on MRS agar media and incubated for 72 h at 37^0C. The colonies on the agar plates were counted by colony counter (Chen *et al.,* 2005).

Sensory Evaluation of Probiotic Ice Cream

Different treatments of ice cream samples were evaluated by 5 trained panellists using a 9 point hedonic scale for the properties such as colour and appearance, flavour and taste, body and texture, and overall acceptability. The samples were served randomly to the panellists.

Statistical Analysis

The data were subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS), the 15^{th} version. Differences between means were tested using Duncan's, (1951) multiple comparison tests and the significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

pH

The mean pH values of the different treatments decreased from initial day to 90 days of storage period at -20°C (Fig. 1).

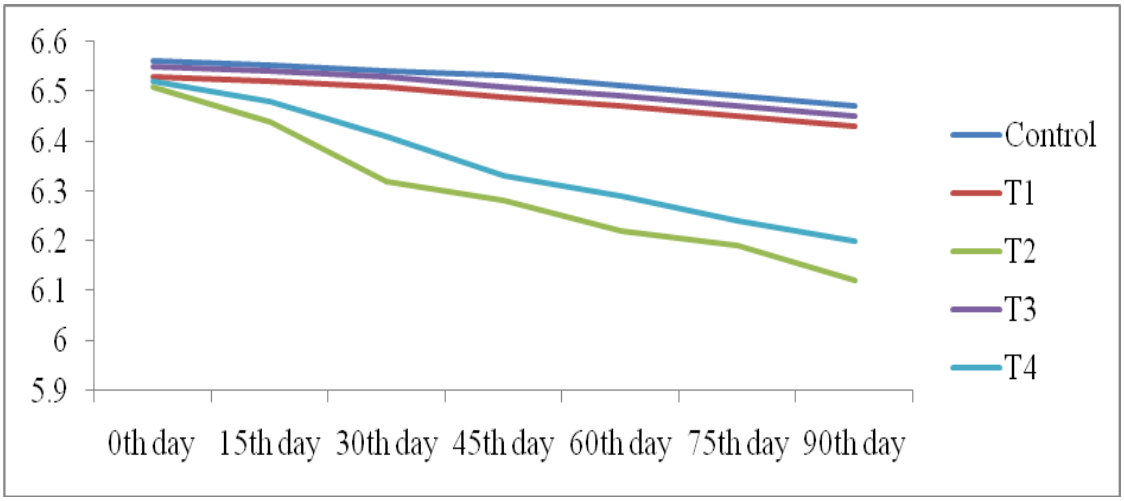

Figure 1: Mean pH values of encapsulated and non encapsulated probiotic strains in ice cream during 90 days storage at -20°C

The non encapsulated strains of *L. helveticus* 194 (T_2) and *B. Bifidum* 231 (T_4) lowered the pH of ice cream significantly ($P<0.05$) when compared with encapsulated strains of *L. helveticus* 194 (T_1) and *B. Bifidum* 231 (T_3). Lowest pH was observed in T_2 followed by T_4. These results are in agreement with Javed and Nadeem, (2011) who reported that *Lactobacillus* spp. are good acid producers compared to the *Bifidobacterium* spp. which might be the reason for low pH. Turgut and Cakmakci, (2009) also reported that ice cream mixes containing *Lactobacillus* spp had significantly ($P<0.05$) lowered pH compared to *Bifidobacterium* spp.

Titratable Acidity

The overall mean percent titratable acidity values of the treatments increased from initial day to 90 days of storage period at -20°C (Table 1).

Table 1: Mean Titratable acidity (% Lactic acid) values of encapsulated and non encapsulated probiotic strains in ice cream during 90 days storage at -20°C

Treatments	0^{th} day	15^{th} day	30^{th} day	45^{th} day	60^{th} day	75^{th} day	90^{th} day
Control	0.23	0.24^{b}	0.25^{b}	0.26^{c}	0.27^{c}	0.28^{c}	0.29^{c}
T_1	0.25	0.26^{b}	0.27^{b}	0.28^{c}	0.29^{c}	0.30^{c}	0.31^{c}
T_2	0.27	0.28^{a}	0.30^{a}	0.33^{a}	0.35^{a}	0.36^{a}	0.38^{a}
T_3	0.24	0.25^{b}	0.26^{b}	0.27^{c}	0.28^{c}	0.29^{c}	0.30^{c}
T_4	0.26	0.27^{ab}	0.28^{ab}	0.29^{b}	0.31^{b}	0.32^{b}	0.33^{b}

The non encapsulated strains of *L. helveticus* 194 (T_2) and *B. bifidum* 231 (T_4) showed significant higher titratable acidity values than encapsulated strains of *L. helveticus* 194 (T_1) and *B. Bifidum* 231 (T_3). It may be due to the fermentation of available prebiotics and lactose by free cells (none encapsulated) wherein the encapsulated cells, ability to ferment the substrate might be restricted due to coating with alginate. Higher titratable acidity values were observed in non encapsulated *L. helveticus* than non encapsulated *B. bifidum.* These results are consistent with observations of Akalin *et al.,* (2008) who reported that the higher acidity values of *Lactobacillus* spp. than *Bifidobacteium* spp. are due to high acid production property of *Lactobacillus* spp. Turgut and Cakmakci, (2009) also reported that ice cream mixes containing *L.acidophilus* had significantly ($P<0.05$) higher acidity values over *B. bifidum*.

Microbiological Analysis

Effect of Freezing of Probiotic Ice Cream Mix: The encapsulated probiotics strains showed marginal reduction in the viable counts compared to non encapsulated probiotics strains of same organism after the freezing process (Table 2). These results are in agreement with Shah and Ravula, (2001) and Haynes and Playne, (2002) who reported that the entrapped cells survived freezing better than free cells ($P<0.05$) when compared within the same strain. Homayouni *et al.* (2008) observed a decline in bacterial counts due to freezing process, which might be due to the freeze injury of cells leading to the death of the cells. The mechanical stress of the mixing and freezing process like scraping of the cylinder wall by the blades of the freezer and also the incorporation of oxygen into the mix may have resulted in further decrease of bacterial count. Hekmat and Mc Mohan, (1992) also reported that the freezing process caused the reduction of one log cycle in viable counts of *L. acidophillus.*

Table 2: Viable counts ($\log_{10}$ cfu/g) of encapsulated and non encapsulated probiotic microorganisms during freezing

Treatments	**T1**	**T2**	**T3**	**T4**
Before freezing	8.77	8.60	8.90	8.85
After freezing	8.59	8.16	8.67	8.23

Encapsulation of probiotics seems to offer protection during freezing process to maintain the viability of cells in ice cream.*Viability of Probiotic Organisms during Frozen Storage in Ice Cream.*
A gradual decrease in total viable counts was observed in all treatments of ice cream (Fig. 2). Encapsulated *L.helveticus* 194 (T_1) showed significantly ($P<0.05$) higher viable counts than non encapsulated *L.helveticus* 194 (T_2) from initial day to 90 days of frozen storage. Similarly encapsulated *B.bifidum* 231 (T_3) showed significantly ($P<0.05$) higher log counts than non encapsulated *B.bifidum* 231 (T_4).
In case of non encapsulated *L.helveticus*, the cell numbers dropped substantially (2.1 log numbers) by 90 days of storage at -20°C. The *B.bifidum* showed a decrease of 1.9 log units in the non encapsulated state after 90 days similarly the above strains when they were encapsulated, showed a decrease of 0.63 and 0.61 log units respectively. The results obtained are in conformity with Homayouni *et al.*, (2008) who demonstrated that the encapsulated cells required longer time to decrease one log cycle in viable counts. Therefore, microencapsulation of probiotic bacteria can increase the viability of probiotics during storage of ice cream.

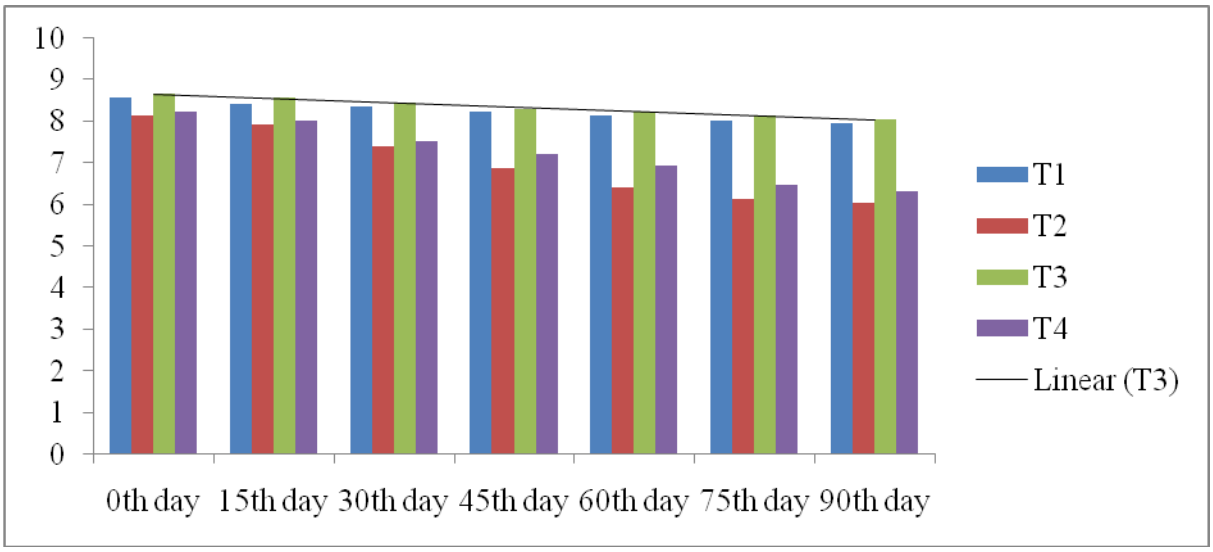

Figure 2: Mean probiotic bacterial counts (log_{10} cfu/g) of encapsulated and non encapsulated probiotic strains in ice cream during 90 days storage at -20°C

Shah and Ravula, (2000) also reported that microencapsulation improved the viable counts of *Lactobacillus acidophilus MJA1* and *Bifidobacterium spp.BDBB2* compared to free cells in frozen fermented dairy desserts stored for 12 weeks. The viable counts of probiotics are higher when they were encapsulated in sodium alginate than not encapsulated. Microencapsulation of probiotic organisms protected their viability in ice cream significantly during freezing process and its frozen storage.
Sensory Evaluation
The sensory scores of the synbiotic ice cream samples for colour flavour and taste, body-texture and overall acceptability showed that the addition of free and encapsulated probiotics had no effect on sensory properties of ice cream. Total evaluation in term of colour, texture and flavour of all samples were good and did not have any marked off-flavour during the storage period.

Conclusion

The present study demonstrates that encapsulation of probiotic organisms had a significant effect in protecting the viability of the organisms both during freezing and during 90 days of frozen storage in ice

cream. Co-encapsulation of probiotics along with prebiotics protected and maintained the viability of cells at optimum levels (10^7cfu/g) as recommended by FAO/WHO. Further, the addition of encapsulated probiotics in ice cream did not show any significant effect on sensory quality of the product.

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