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IMPROVING THE SURVIVAL OF PROBIOTICS IN ICE-CREAM USING MICROENCAPSULATION

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ABSTRACT

Viability and stability of probiotics in functional dairy foods have been a major concern, because a high number of organisms are needed to confer health benefits to the ultimate end users- consumers. In this regard, an investigation was done to evaluate the survivability of two proven probiotics viz., Lactobacillus acidophilus (LA-5) and Lactobacillus rhamnosus (NCDC-18)in ice cream by employing microencapsulation technique. Microencapsulation was done by extrusion and emulsion methods by using two different combination of wall materials like sodium alginate + starch and sodium alginate + starch + whey protein concentrate. The survival of L.acidophilus(LA-5) and L.rhamnosus (NCDC-18) were monitored during the storage period of 120 days at −23°C. The probiotic viability between 10^7 and 10^8 cfu/g at the end of three months of storage of ice cream (which is the normal shelf life of ice cream) has been achieved in all encapsulation procedure employed in the present study. Among all treatments, treatment-IV and treatment-V (emulsion method of encapsulation using sodium alginate + starch and sodium alginate + starch + whey protein concentrate as wall materials) showed good viability in probiotic strains and different environmental condition. It is concluded that microencapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended period of shelf-life without affecting the physic chemical and sensorial attributes.

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

I .INTRODUCTION

Ice cream is a delicious and 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.

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. Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host [1].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. Among the most used organisms are those belonging to the genera of *Lactobacillus* which is believed to have beneficial effects on human health [2]

Considering 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.

Probiotic dairy products development 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 [3]. Microencapsulation 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 improve the survival of probiotic bacteria in frozen desserts [4].

The objective of this study was to evaluate the survival of microencapsulated and free probiotic culture in ice cream over a period of 120 days storage at ⁻23°C by using sodium alginate, starch and whey protein concentrate as wall materials in the beads.

II. MATERIALS AND METHOD

The experimental design of different treatments of unencapsulated and encapsulated probiotic ice cream were as follows

Probiotics	**Un encapsulated probiotic ice cream**	**Encapsulated probiotic ice cream**			
		Methods			
		Extrusion		**Emulsion**	
		Sodium alginate + starch	**Sodium alginate+ starch+whey protein concentrate**	**Sodium alginate+ starch**	**Sodium alginate+ starch+whey protein concentrate**
Lactobacillus acidophilus (LA-5)	UPIA-I	EPIA -II	EPIA -III	EPIA -IV	EPIA -V
Lactobacillus rhamnosus (NCDC-18)	UPIR-I	EPIR -II	EPIR -III	EPIR -IV	EPIR -V

2.1 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,themix ingredients were homogenized as described by Arbuckle [5] 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., *L.acidophilus(LA-5)* and *L. rhamnosus* (NCDC-18)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&Mcmahon1992). 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 freezed at – 4 to – 5^0C and stored at – 23^0C where the ice cream was hardened.

2.2 Enumeration of free and encapsulated probiotics

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. Bacterial counts were enumerated before and immediately after freezing as well as at the end of every 30 days until 120 days of storage at −23°C.

Enumeration of probiotic bacteria was achieved as described by Haynes and Playne[6]. All enumerating plates of *L.acidophilus(LA-5)* and *L. rhamnosus* (NCDC-18)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*et al.*[7].

2.3 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. The overrun of the final product was determined using the following formula [8]

2.4 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.*[8].

2.5 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).

III. RESULTS AND DISCUSSION

3.1 Physico-chemical characteristics

The different treatments of both unencapsulated and encapsulated probiotic ice creams showed relatively low reduction in pH when compared with the control. The whipping ability of unencapsulated and encapsulated probiotic ice cream showed no significant differences between control, unencapsulated and encapsulated treatments, regarding overrun, there were no significant differences observed between different treatments of ice cream. Among different treatments of unencapsulated and encapsulated probiotic ice cream, there was no significance difference was observed with regard to meltdown time at a particular storage period and the mean value of meltdown time was gradually increased as the storage period increased.

3.2 Survivability of free and encapsulated *L.acidophilus* (LA-5) in probiotic ice cream during different storage period

The results showed that in unencapsulated probiotic ice cream samples, there was no significant viability changes up to 30 days storage but after that there was a significant reduction in the viability of *L. acidophilus* (LA-5) at every 30 days of refrigerated storage period ($P<0.01$). But, encapsulated treatment samples EPIA-II and EPIA-III showed a reduction in the viability of one log unit between 30 and 60, 90 and 120 days of storage. (Table-1)

Further, EPIA-IV and EPIA-V samples showed only one log unit reduction in viability between 30 and 60 days of storage period, after that there was no significant reduction in the viability up to 120 days of storage. This can also be substantiated by the findings of Heenan *et al.* [9], who found that there was no marked reduction in the initial population (10^7-10^8 cfu/g) of *Lactobacillus* spp and *Bifidobacterium* spp (except *L. paracasei)* in probiotic ice cream throughout the storage period of 28 weeks at -20°C .Salem *et al*. [10] documented that during 12 weeks of storage, the viability of *L. acidophilus* decreased by 2.23 log cfu/g and they also reasoned out that the decline in bacterial number was due to freezing of all cells resulting in the death of some cells, mechanical stresses of mixing and freezing process and also incorporation of oxygen into the mix and Zanjani*et al*.[11] reported that *Lactobacillus sp.* survived better by encapsulation using emulsion technique with calcium alginate-starch as wall material.

This study noted that all the treatments with probiotic strain of *L.acidophilus* (LA-5) showed better viability of above 7 log units at the end of 120 days of storage. Among the treatments, EPIA-IV and EPIA-V showed maximum viability up to 120 days of storage and these treatments were selected as superior for further viability study. This might be attributed to the smaller sized emulsion beads (when compared to extrusion beads), which might have escaped from churning of ice cream mix. Further, the whey protein concentrate added as wall material might have givenadded protection to probiotics during the storage period.

Table-1.Viability of free and encapsulated *L.acidophilus (LA-5)* in probiotic ice cream during different storage period (log_{10}cfu/ml).

Treatment	Storage period (in days)					
	0 day	**15 days**	**30 days**	**60 days**	**90 days**	**120 days**
UPIA-I	$9.84^{Ba} \pm 0.044$	$9.74^{Ba} \pm 0.046$	$9.13^{Ab} \pm 0.072$	$8.35^{Ac} \pm 0.045$	$7.34^{Bd} \pm 0.052$	$6.90^{Ce} \pm 0.013$
EPIA-II	$9.64^{Aa} \pm 0.022$	$9.53^{Aa} \pm 0.054$	$9.18^{Ab} \pm 0.064$	$8.38^{Ac} \pm 0.052$	$8.09^{Ac} \pm 0.016$	$7.89^{Ad} \pm 0.091$
EPIA-III	$9.65^{Aa} \pm 0.024$	$9.59^{Aa} \pm 0.043$	$9.17^{Ab} \pm 0.057$	$8.45^{Bc} \pm 0.043$	$8.01^{Ad} \pm 0.076$	$7.95^{Ae} \pm 0.087$
EPIA-IV	$9.72^{Aa} \pm 0.031$	$9.64^{Aa} \pm 0.041$	$9.31^{Bb} \pm 0.053$	$8.63^{Cc} \pm 0.049$	$8.56^{Cc} \pm 0.016$	$8.52^{Bc} \pm 0.089$
EPIA-V	$9.77^{Aa} \pm 0.036$	$9.69^{Aa} \pm 0.039$	$9.33^{Bb} \pm 0.074$	$8.75^{Dc} \pm 0.073$	$8.65^{Cc} \pm 0.077$	$8.65^{Bc} \pm 0.083$

Different lower case superscripts in a row differ significantly at P>0.05

Different uppercase superscripts in a column differ significantly P<0.01

3.3 Survivability of free and encapsulated *L.rhamnosus* (NCDC-18) in probiotic ice cream during different storage period

Ice cream samples containing probiotics in unencapsulated form showed no significant viability changes up to 15 days of storage and after that there was a significant reduction in the viability of *L.rhamnosus* (NCDC-18)in 30, 90 and 120 days of refrigerated storage period. (Table-2)

Among different treatments, EPIR-IV and EPIR-V samples showed one log unit reduction in the viability between 15 and 30 days of storage period, after that there was no significant reduction in the viability up to 120 days of storage. These findings concurred with the results of Fahimdanesh *et al*. [12], who reported that microencapsulation with resistant starch enhanced the survival of *L.rhamnosus* as compared to free cells in the mayonnaise sauce up to 30 days of storage.

This is also strengthened by the results of Ozer*et al*. [13], who concluded that the viability of *L.rhamnosus* (NCDC-18) encapsulated in sodium alginate by either an extrusion or emulsion technique in white-brined cheese was found to be effective in keeping the numbers of probiotic bacteria higher than the level of the therapeutic minimum ($>10^7$ cfu/g) required. Godward and Kailasapathy [14] studied the preparation of ice cream with incorporation of probiotic bacterial culture *B. infantis*-1912in the forms of free, freshly encapsulated and freeze dried cultures and concluded that encapsulated cells survived better than free cells in ice cream during a storage period of 24 weeks at -20°C.

EPIR-IV and EPIR-V showed higher viability during storage period, Further, when compared with the viability of *L.acidophilus* (LA-5), both the unencapsulated and encapsulated forms of *L.rhamnosus* (NCDC-18) strain showed lesser viability this may be due to the lower resistance of the *L.rhamnosus* (NCDC-18) to the churning and freezing and storage conditions in the ice cream manufacture and storage. Added to that, sodium alginate and whey protein concentrate might have given more protection to probiotic strains from adverse environmental conditions during storage period.

Table -2. Viability of free and encapsulated *L.rhamnosus* (NCDC-18)in probiotic ice cream during different storage period (log_{10}cfu/ml).

Treatment	Storage period(in days)					
	0 day	15 days	30 days	60 days	90 days	120 days
UPIR-I	$9.74^{Aa} \pm 0.062$	$9.57^{Ab} \pm 0.045$	$8.45^{Ac} \pm 0.065$	$8.23^{Ad} \pm 0.045$	$7.41^{Ce} \pm 0.048$	$6.76^{Cf} \pm 0.017$
EPIR-II	$9.77^{Aa} \pm 0.051$	$9.52^{Ab} \pm 0.052$	$8.47^{Ac} \pm 0.069$	$8.25^{Ad} \pm 0.054$	$8.02^{Ae} \pm 0.023$	$7.82^{Af} \pm 0.085$
EPIR-III	$9.79^{Aa} \pm 0.085$	$9.52^{Ab} \pm 0.047$	$8.49^{Ac} \pm 0.054$	$8.24^{Ad} \pm 0.059$	$8.05^{Ae} \pm 0.056$	$7.82^{Af} \pm 0.018$
EPIR-IV	$9.75^{Aa} \pm 0.076$	$9.57^{Ab} \pm 0.051$	$8.67^{Bc} \pm 0.066$	$8.57^{Bc} \pm 0.078$	$8.53^{Bc} \pm 0.046$	$8.41^{Bc} \pm 0.064$
EPIR-V	$9.79^{Aa} \pm 0.064$	$9.62^{Ab} \pm 0.054$	$8.79^{Bc} \pm 0.061$	$8.65^{Cc} \pm 0.052$	$8.62^{Bc} \pm 0.071$	$8.44^{Bd} \pm 0.089$

Different lower case superscripts in a row differ significantly at P>0.05

Different uppercase superscripts in a column differ significantly P<0.01

IV. CONCLUSION

In the present study, the probiotic viability between 10^7 and 10^8 cfu/g at the end of three months of storage of ice cream (which is the normal shelf life of ice cream) has been achieved in all encapsulation procedure employed. This viable cell number is higher than that of the recommendation of International Dairy Federation (10^7cfu/g). Among all treatments, the treatment-IV and V showed good viability for the above twoprobiotic strains during different storage periods without altering the physico-chemical and sensorial attributes of probiotic ice cream. It is concluded that probiotic survivability in ice cream can be significantly improved by microencapsulation technique.

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