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PREPARATION OF HEALTH BENEFICIAL PROBIOTIC SOYA ICE -CREAM AND EVALUATION OF QUALITY ATTRIBUTES

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ABSTRACT

Probiotic soya ice cream was prepared by using food grade bacteria *Lactobacillus paracasei* S1(MF443873) and *Lactobacillus rhamnosus* KC6 (MF443875). Bacteria have been encapsulated in milk and sodium alginate beads to improve their viability in soya ice -cream. Viable cell number of entrapped probiotic isolates was observed with calcium chloride concentration of 0.1M at pH 7.0, hardening time of 8h. Morphological characterization of microencapsulated beads was done by using SEM. Probiotic soy ice-cream was stored at -20ºC for 30 days and was investigated for sensory qualities, microbial count and physicochemical characteristics. Microencapsulated probiotic ice-cream was found more stable during storage as compared to those containing free probiotic microorganisms. As far as physico-chemical characteristics of probiotic enriched soy ice-cream is concerned, though changes in quality attributes viz. pH, ascorbic acid, lactic acid, total soluble solid and antioxidant content during storage interval have been observed but these changes were statistically non- significant.

Keywords: Lactic acid bacteria, Probiotic, Encapsulation, Soya milk

INTRODUCTION

Scientific investigations revealed that maintenance of healthy gut microflora may offer protection against numerous gastrointestinal disorders **(Veldes *et al.*, 2018; Gautam and Sharma, 2014)**. Lactic acid bacteria is characterized as GRAS and has been proved for its probiotic potential **(Gulfam, 2017; Gautam and Sharma, 2015)**. Probiotics are "live microorganisms which when administered in adequate amount confer a health benefit to the host (**FAO, 2007)**. There is an upsurged curiosity of food scientists throughout the world to add these health beneficial bacteria in food preparations so as to make food items more nutritionally rich. The probiotic market has grown rapidly, both for foods and supplements intended to enhance wellness in healthy individuals, and for preparations of the dietary management of disease **(Simone, 2019)**. Global probiotic industry is heading towards making various probiotic formulations and at a pace to increase turnover year wise. Probiotic market is segmented by Probiotic food/drinks, dietary supplements, animal feed etc. The ongoing COVID-19 pandemic has had its positive impact on probiotic market as there is an upsurge in demand of food, drinks and supplements that have potential to boost immune system. Keeping in view the above, present investigation was carried out to make nutritionally rich probiotic soya ice cream. Soya bean is high in fibre and protein content, its low in saturated fat, lactose free, cholesterol free and a good source of omega-3-fatty acid. It is a powerhouse of health benefits, it maintains diabetes, improves blood circulation, manage cancer risk and boost heart health etc. Ice -cream is a sweetened frozen desert prepared with water, ice, milk fat, milk protein and sugar. It is widely consumed throughout the world as a creamy delight. Ice cream by virtue of milk as its major ingredient has nutritional properties but own no health benefits (Salem *et al*. 2005). Recently, an increased demand from consumers for healthier and better functional food has led to produce ice cream containing special ingredients with recognized nutritional and physiological properties such as probiotics and natural antioxidants. This study focuses on the importance and safety of probiotic soya ice- cream. Consumption of ice cream containing probiotics can reduce the bacterial level in the mouth responsible for tooth decay, inhibit the growth of potential pathogens, improve intestinal microflora and activate the immune system. Developing countries where water borne disease are prevalent, eating 1-2 tablespoon of probiotic ice cream can keep water borne disease at bay **(Amul, 2007).** Therefore, keeping in mind the health benefits of Soya milk as well as lactic acid bacteria, combination of these two are used to prepare health beneficial probiotic ice-cream by using microencapsulation technique. The objective of our study was microencapsulation of potential probiotic Lactic acid bacteria and to prepare soya based ice-cream by using encapsulated lactic acid bacteria.

MATERIAL AND METHODS

Bacterial isolates *Lactobacillus paracasei S1* (Accession no MF443873) and *Lactobacillus rhamnosus* KC6 (Accession no MF443875) having probiotic characters were used to prepare probiotic soya-based ice cream.
These isolates were used in encapsulated as well as in free form.

Microencapsulation of probiotic bacterial isolates

Encapsulation parameters: **(Rodrigues, *et al.* 2011; Frakolaki *et al.*, 2022; Bajwa, 2018).**

1. ***Sodium alginate:***
   Sodium alginate was obtained from Hi-media. Different combinations of sodium alginate were used for the preparation of encapsulating matrix i.e. 0.75%, 1%, 1.5%, 1.8% and 2% w/v.
2. ***Calcium chloride***:
   Different combinations of calcium chloride wereusedi.e.0.1, 0.2, 1M
3. ***pH:***
   The effect of pH in the range of 3.0, 4.0, 5.0, 6.0, 6.5, 7.0 and 9.0 was evaluated on the preparation of encapsulating beads.
4. ***Hardening time***
   The hardening of the encapsulated beads was evaluated over a time period of 5 min, 30 min, 1 h, 2 h and 8 h.

Microencapsulation using Milk and Sodium Alginate

Preparation of probiotic cell suspensions:

L. paracasei S1 and *L. rhamnosus* KC6 were cultured on MRS broth at 37°C for 24h. Cells were harvested by centrifugation at 1500 rpm for 5 min at low temperature (4 °C) and the cell pellet was washed twice with sterile saline solution. The cell suspension of each bacterium was used for microencapsulation. Fresh cell suspensions were prepared for each experiment and enumerated by pour plating on MRS media plates.

Preparation of different microspheres formulations:

Different combinations of milk and sodium alginate were used for the preparation of encapsulated beads, and they are as follows:

1. A_1M_1Alginate: Milk=1/1v/v alginate concentration1%
2. A_1M_2Alginate: Milk=1/2v/v alginateconcentration1%
3. A_1M_3Alginate: Milk=1/3v/v alginate concentration1%
4. A_1M_4Alginate: Milk=1/4v/v alginate concentration1%
5. $A_{1.5}M_4$Alginate: Milk=1/4v/v alginateconcentration1.5%
6. A_2M_4Alginate: Milk=1/4v/v alginate concentration 2%

Preparation of encapsulated beads using the extrusion method (Zhou *et al.* 2005, *Bajwa* (2018)

Microencapsulation using pure milk and sodium alginate were sterilized for 15 min at110^{0}Cand121ºC, respectively. Sodium alginate milk microspheres were made usinga 0.11 mm needle into sterile calcium chloride, which was stirred at 200 rpm by a magnetic stirrer. The distance between the needle and the calcium chloride solution was fixed at 3cm. Beads were immediately formed when the mixture came in contact with the calcium chloride solution. The beads were then allowed to stand for 30 -120 min for gelification. The beads were then rinsed with sterile 0.1% peptone solution at 4°C.

Release and enumeration of the encapsulated cells

Encapsulated cells were released from the beads before enumerating. For the beads obtained with the extrusion method, 1 g of particles were taken and added into 9 ml of potassium phosphate buffer (0.1M, pH 7.0). It was then blended gently for 10 min. An aliquot of 1 ml of the suspension obtained was serially diluted for plating. Released cells were enumerated by spreading 100 µL of the diluted suspension into MRS and incubating for 48 hat 37°C anaerobically.

Morphological characterization of microencapsulated beads

Scanning Electron Microscopy (SEM) Kim (2008)

The morphological characterization of alginate beads was determined by SEM. For this sample obtained after the pre-treatment were centrifuged at 10,000 rpm for 10 min. The beads so obtained were washed twice with ethanol and again centrifuged. Thesampleswerewashedtwicewithbutylalcoholandagaincentrifuged.Samples were then dried and were attached to a SEM stub with silver plate. The mounted samples were then spatter coated with gold using fine coat, JEOL ion sputter, Model JFC-100Thegoldcoatedstubswereexaminedatdifferentmagnificationunderscanningelect ron microscope; model Hitachi S-3400N field emission SEM (Hitachi High-Tech, Japan).at10 kV.

Preparation of Functional Soy Ice-Cream enriched with potential probiotic isolates

Ingredients

Soy-bean grains	250g
Autoclaved distilled wáter	2000 ml
Table Sugar	17%
Gaurgum	0.6%
L.paracasei S1 (free)	10^8CFU/ml
***L.rhamnosus* KC6 (free)**	**10^8CFU/ml**
L.paracasei S1+*L.*rhamnosusKC6 (free)	10^8CFU/ml
L.paracasei S1 (encapsulated)	10^8CFU/m
L.rhamnosus KC6 (encapsulated)	10^8CFU/ml

Steps in preparing Soya ice cream

1. Soyabeans (250g) washed three times
2. Soaked in (2000 ml) water for 14 h at room temperature
3. Excess water was then drained off and the shells were removed.
4. The swollen beans blended with 250 ml of boiling water in a laboratory blender for 5 min
5. Slurry (2000 ml sterilized water)-Filtration
6. Soya milk
7. Soyamilk reheated at 80°C for 10 min.
8. Chilled at 4^{0}C prior to making ice cream
9. Addition of sugar (17 %)
10. Addition of gaur gum (0.6 %)
11. Heat treatment (80^{0}C, 10 min)
12. Cooling at room temperature
13. To ensure equal concentration of cells are added (MRS broth) inoculated with *L.paracasei* S1 (a) and *L rhamnosus* KC6 (10 ml each) separately
14. Centrifuged at 10,000 rpm for 5 min
15. 1ml of washed cells serially diluted 10^8 CFU/ml (free)
16. 1ml of washed cells encapsulated using (sodium alginate and milk matrix)

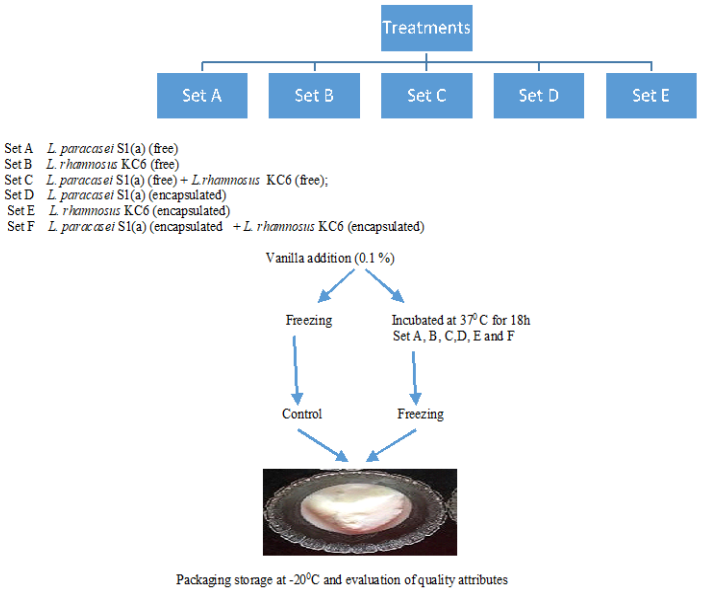

Figure 1 Preparation of functional soy ice cream

Sensorial evaluation

Sensorial evaluation of each sample was done in terms of appearance, texture, flavour and overall acceptability. Nine-point hedonic scale method as given by **Joslyn and Amerine, (1964)** was followed for conducting the sensory evaluation of probiotic food products. The panel of 10 judges (5 males and 5 females of age group 20 to 50 years) was selected to evaluate the products for sensory parameters such as appearance, flavor, texture, taste and overall acceptability depending upon the type of product. Efforts were made to keep the same panel for sensory evaluation throughout the course of study.

Microbial evaluation during storage

The colony counts of *L. paracasei* S1 and *L. rhamnosus* KC6 (free and encapsulated cells) was observed during storage period of 30 days after the interval of every 7 days by standard spread plate count method. MRS agar was used to enumerate lactic acid bacteria while nutrient agar was used to enumerate other bacteria.

Physicochemical changes during storage

pH: pH of each sample was measured using pH meter at an interval of 0, 7, 14, 21 and 30days.

Total soluble solids: TSS was measured by placing 1-2 drops of sample on the prism of a hand refractometer. For 0, 7, 14, 21 and 30 days the results were expressed as ºB **(Ranganna, 1995).**

Acidity in terms of lactic acid acid: An aliquot of the sample prepared was diluted with recently boiled distilled water. 2-3 drops of 1% phenolphthalein solution was used as an indicator and titration was done with 0.1N NaOH. Titer value was noted and calculations were done as percent anhydrous lactic acid.

$$\text{Titrable acidity} = \frac{\text{Titre x Normality of alkali} \times \text{Volume made up} \times \text{Equivalent weight}}{\text{Volume of sample taken} \times \text{volume of taken} \times 100} x\ 100$$

Ascorbic acid*:* Ascorbic acid was determined as per AOA C (1995) method for 0, 7, 14, 21 and 30 days.

Assay:

An aliquot of 1 ml of sample was diluted with 10 ml of 3% HPO_3 followed by centrifugation. Aliquot of the sample was titrated with the standard dye to a pink end point which persisted for 15 sec. Titration was done rapidly and preliminary determination of the titre was done.

Calculations

The ascorbic acid content of the sample was determined by following method:

$$\text{mg of ascorbic acid per 100 g or ml} = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken} \times \text{weight of sample taken}} x\ 100$$

Free radical scavenging activity (FRSA) (William *et al.* 1995)

Free radical scavenging activity was measured as per the method of **William *et al*. (1995).** DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used as a source of free radical. An aliquot of 3.9 ml of $6x10^{-5}$mol/l DPPH in methanol was taken in a cuvette with 0.1 ml of sample extract and the decrease in absorbance was measured at 515 nm for 30 min or until the absorbance become steady. The remaining DPPH concentration was calculated using the following equation:
Free radical scavenging activity (%) $=Ab_{(b)}-Ab_{(s)}$
Where, $Ab_{(b)}$=Absorbance of blank, $Ab_{(s)}$=Absorbance of sample

Statistical analysis

Data pertaining to the physicochemical attributes of probiotic product was analyzed by completely randomized design (CRD) as described by **Mahony (1985).**

RESULTS AND DISCUSSION

Microencapsulation of Probiotic Strains

Microencapsulation using Milk and Sodium Alginate

Bacterial isolates *L. paracasei* S1 isolated from Sidra- sun dried fish and *L. rhamnosus* KC6 isolated from curd. These isolates were tested for probiotic characteristics viz. bile salt tolerance, haemolytic activity, hydrophobicity, antagonistic activity, autoaggregation activity, coaggregation, exopolysaccharide production and β-galactosidase assay. Cumulative probiotic potential of *L. paracasei* S1 is 95.83 % and *L.rhamnosus* KC6 is 100 %. These probiotic strains were used to prepare nutritionally rich probiotic soya-based ice cream. There is low viability of probiotic microorganisms in food products **(Gilliland and Speck, 1977).** Microencapsulation has been investigated to protect the microorganisms in the product's environment and improve their survival. In the present investigation microencapsulation of potential probiotic microorganisms i.e. *L. paracasei* S1 and *L. rhamnosus* KC6, has been done for improving their viability in functional food products. Extrusion technique is followed for encapsulation of potential probiotic microorganisms for their use in preparation of probiotic soya ice-cream.
Extrusion method was used to encapsulate potential probiotic microorganisms, different combinations were prepared with milk and sodium alginate for the preparation of beads i.e. (A_1M_1, A_1M_2, A_1M_3, A_1M_4, $A_{1.5}M_4$ and A_2M_4) with increase in the alginate concentration in microspheres the sphericity of microspheres increased. Higher alginate concentration in microspheres like $A_{1.5}M_4$ and A_2M_4 led to better sphericity of microspheres. Microspheres (A_1M_4) with low alginate concentration had soft texture and flat beads. A_2M_4 with high alginate concentration had round and stable beads and was further used in the study. Viable cell number of entrapped probiotic isolates i.e. *L. paracasei* S1 and *L. rhamnosus* KC6, was observed at pH7.0, with calcium chloride concentration of 0.1M and hardening time of 8h. It was observed that cell entrapment for *L. paracasei* S1 (9.63 CFU/g) and for *L. rhamnosus* KC6 (9.34 CFU/g). This study is in accordance to the study of Shi *et al.* (2013), *Lactobacillus bulgaricus* was encapsulated in alginate–milk microspheres prepared by extrusion method. The viability of encapsulated *L. bulgaricus* of microspheres could be kept more than 8 log CFU/g after 120 min incubation. The viability of encapsulated *L. bulgaricus* after 12 h of incubation was observed to be 9.98 log CFU/g microspheres. **Wurth *et al.* (2015)**, microencapsulated *Lactobacillus* strains in milk-protein-based microcapsules to increase bacterial survival in simulated gastric juice (SGJ).

Morphological characterization of Microencapsulated potential probiotic microorganisms by Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is used usually for the structure analysis. The major scope of the image analysis is to define parameters such as size and shape of the objects which are recognized in an image. SEM visualization is considered as most appropriate because it gives highly magnified, well-focused, high resolution images and is a good technique to verify the presence of microorganisms in the interior of microparticles. The figure 2 shows the appearance of microparticles, in their respective matrix, i.e. 500 µm. The films comprised of sodium alginate and sodium caseinate; tapioca starch, sodium alginate and tragacanth gum; milk and sodium alginate and sodium alginate matrices, maintained their compact, and non-porous fibrous-like structures.

Figure 2 Characterization of Microencapsulated beads by Scanning Electron Microscope. **a**) Section of sodium alginate and milk microcapsules showing full bead. b) Cross section of the sodium alginate and milk beads. c) Internal structure of the sodium alginate milk matrix

The Microcapsules so obtained were round, flattened shape (not completely smooth) without visible cracks or pores on the surface. The microcapsules remained stable without changes in size and morphology. Tubular and star like structure were visible in the microspheres which attributed in the presence of microorganisms, the potential probiotic microorganisms can be seen distributed randomly in the matrix. Similar study has been reported in literature, where **Mahmoud *et al*. (2020)**, had encapsulated lactic acid bacteria within alginate microspheres and evaluated its surface morphology by scanning electron microscopy. Omega-3 fatty acids and probiotic bacteria were co-encapsulated in a single whey protein isolate (WPI)–gum Arabic (GA) complex microcapsule, and their morphology was examined by scanning electron microscopy **(Eratte *et al.*, 2015).**

Preparation of functional Soya Ice-Cream using *L. Paracasei* S1 and *L. rhamnosus* KC6

In the present investigation, health promoting soya ice cream with additional health benefits (nutraceuticals) have been formulated by adding potential probiotic strains in different combinations as given in materials and methods (Figure 1 & Figure 3). The potential probiotic isolates were encapsulated using milk and sodium alginate matrix. The ice soy ice-cream was prepared by fermenting *L. paracasei* S1(a) and *L. rhamnosus* KC6 10^8 CFU/ml in seven different sets. After fermentation for 37ºC for 18 h, all the sets were kept in refrigeration at -20ºC for 30 days for further evaluation i.e. pH, TSS, titratable acidity and ascorbic acid. Nutritional chart of probiotic functional soy ice-cream enriched with potential probiotic isolates had been presented in (Table1 & Table 2). The product was found rich in antioxidants (61.76%-63.90%), proteins (3.51%), carbohydrates (12.17g), titratable acidity (0.16-0.29%) and ascorbic acid (7.0-9.0 mg/100g).

Figure 3 Probiotic Enriched Soya Ice Cream

Table 1 Nutritional Evaluation chart of probiotic enriched Soy-Ice cream

Nutritional factsper100ml	Samples						
	Control	Set A	Set B	Set C	Set D	Set E	Set F
Fat (%)	2.5	2.51	2.50	2.52	2.50	2.50	2.50
Protein (%)	3.3	3.42	3.44	3.5	3.51	3.42	3.34
pH	6.74	6.72	6.71	6.77	6.74	6.66	6.71
Antioxidants (%)	61.76	60.80	61.80	60.85	62.87	63.89	63.90
Carbohydrates (g)	12.11	12.15	12.16	12.12	12.15	12.17	12.16

Legend: Set A: *L. paracasei*S1 (free); Set B: *L. rhamnosus* KC 6 (free); Set C: *L. paracasei* S1 + *L. rhamnosus;* KC6 (free); Set D: *L. paracasei* S1(encapsulated); Set E: *L. rhamnosus;* KC6 (encapsulated) Set F; *L. paracasei* S1+*L.rhamnosus*KC6 (encapsulated)

Functional foods/nutraceuticals are defined by Expert Report of Institute of Food Technologists of USA in 2004 and American Dietetic Association in 2009 as food and food components (bio active substance) that provide a health benefit beyond basic nutrition including the prevention and/or treatment of a disease **(Ali, 2021)**. Gut microbiota especially probiotics transform and influence the bioavailability and effects of many bioactive components in GI tract and their metabolic products also inhibit pathogenic bacteria **(Indira M, 2019)** while some stimulate the growth of beneficial bacteria, exerting prebiotic-like effects **(FAO/WHO, 2007).** Interactions between functional food components, such as prebiotics and probiotics

have various consequences on human health (improving gut microflora, immune responses, reducing lactose intolerance, GI infections etc.) **(Ballini, 2023).**

Sensorial Evaluation

Soya ice cream enriched with potential probiotic isolates, (both free and encapsulated) were assessed by 10 panelists using 9 point hedonic scale for some sensory parameters (i.e. appearance, flavour, texture and overall acceptability), as described by *Joslyn* and Amerine (1964). In a sensory evaluation, control was least accepted with a score of 7.5 while Set F had maximum acceptability i.e. 8.55 out of 10. It seems from the results that addition of encapsulated beads had positive effect on sensory properties of the soy ice- cream. Set B, C, D and E showed acceptable effect on sensory attributes of probiotic enriched functional soy ice - cream, as compared to control. The results of above experiment also reflected that the probiotic strains added to the product contributed a significant influence on the overall acceptability of the product.

Changes in quality attributes of probiotic enriched functional soy ice-cream

Probiotic enriched functional soy ice- cream was kept under refrigeration upto 30 days and the quality attributes (pH, total soluble solids, titratable acidity, ascorbic acid and antioxidant activity) of each set were noted on 0, 7th, 14th, 21st and 30th day of storage as described in Table 2. The pH value of probiotic enriched functional soy ice- cream was observed to decrease during storage period because of the sugars which encourage probiotic growth and in turn reduce the pH of the ice-cream during storage period. On 30th day, maximum decrease was observed in Set C (5.38) and minimum in control (6.58). Total, soluble solids (TSS) of probiotic enriched functional soy ice- cream slightly decreased in ^{o}brix values as addition of potential probiotic microorganisms has no effect on the TSS values, initially, TSS value of 10^{o}B was observed for all the sets. On 30th day, maximum decrease was observed in control and Set A while in Set B, C, D and E, very less reduction in ^{o}B was observed. Furthermore, increase of ascorbic acid content in functional soy ice- cream, with potential probiotic isolates, can be explained by the fact that ascorbic acid is an oxygen scavenging ingredient thus promoting anaerobic conditions, which encourage probiotic growth and in return does not allow ascorbic acid to leach out (Gomes & Malcata, 1999). Initially, ascorbic acid content was 6.5 mg/100gin Set E which rose in all the sets except control with maximum in Set F (9.0 mg/100g) and minimum in control (7.0 mg/100g) upto 30th day. Maximum antioxidant activity was observed in Set F (63.90 %) while the lowest value was recorded in control (61.76 %). A continuous increase was observed during storage period in the antioxidant content of probiotic enriched functional soy ice- cream. During the storage of 30 days, Set F contain *L. paracasei* S1 and *L. rhamnosus* KC6 encapsulated was considered the best set as, it showed a significant increase in ascorbic acid(9.0) and antioxidant (63.90) while, statistically, changes in quality attributes of probiotic enriched soy ice-cream during storage period were analyzed by CRD factorial which revealed that changes in quality attributes of the product were non-significant during storage conditions, thus recommending the stability and good quality of product for consumption even after 30 days of storage.

Table 2 Physicochemical characteristics of probiotic enriched functional soya ice cream

	Storage interval (days)																							
	pH						TSS (^{o}B)						Titratable acidity (%)						Ascorbic acid (mg/ml)					
	0	**7**	**14**	**21**	**30**	**Mean**	**0**	**7**	**14**	**21**	**30**	**Mean**	**0**	**7**	**14**	**21**	**30**	**Mean**	**0**	**7**	**14**	**21**	**30**	**Mean**
Set A	6.66	6.45	6.22	6.10	5.88	5.64	10	10	9.9	9.7	8	9.52	0.15	0.152	0.16	0.18	0.24	0.17	7.2	8.0	7.7	8.2	8.5	**7.92**
Set B	6.71	6.65	6.50	6.42	6.38	5.68	10	9.8	10	10	9.2	9.8	0.17	0.18	0.20	0.24	0.28	0.21	7	7.5	8	8.5	8	**7.8**
Set C	6.50	6.37	6.24	5.75	5.38	5.43	10	9.5	9.5	9.	8.5	9.3	0.17	0.18	0.18	0.22	0.28	0.20	7	7.5	8	8.5	8.7	**7.94**
Set D	6.71	6.69	6.64	6.60	6.6	6.64	10	9.8	9.8	9.5	9.7	9.76	0.17	0.17	0.18	0.24	0.29	0.19	7.2	7.5	8	8.5	8.9	**8.02**
Set E	6.70	6.68	6.66	6.63	6.60	6.65	10	10	9.7	9	9	9.54	0.16	0.16	0.18	0.20	0.27	0.21	6.5	7.5	8.2	8.5	8.8	**7.9**
Set F	6.69	6.66	6.54	6.50	6.52	6.58	10	10	10	9.5	9.2	9.74	0.16	0.18	0.19	0.25	0.29	0.18	7.2	7.8	8.4	9	9.0	**8.28**
Control	6.74	6.70	6.66	6.69	6.67	6.69	10	10	10	9	9	9.6	0.15	0.18	0.18	0.20	0.23	0.18	7.5	7.5	7.4	7.2	7.0	**8.02**
Mean	6.67	6.65	6.61	6.5	6.58		10	9.87	9.84	9.38	8.94		0.16	0.17	0.18	0.21	0.26		7.08	7.44	7.40	7.37	7.36	
CD0.05		Treatment (0.09) D (0.11) txd= (0.25)						Treatment (0.09) D (0.11) txd= (0.25)						Treatment (N/A) D (0.22) txd= (N/A)						Treatment (0.10) D(0.12) txd= (0.26)					

Legend: Set A: *L. paracasei*S1 (free); Se B: *L.rhamnosus* KC6 (free); Set C: *L. paracasei*S1 + *L. rhamnosus;* KC6 (free); Set D: *L. paracasei* S1 (encapsulated); Set E:*L.rhamnosus;* KC6 (encapsulated) SetF; *L. paracasei* S1+*L.rhamnosus* KC6 (encapsulated)
CD = Critical difference
D = factors (pH, TSS, Titratable acidity, Ascorbic acid)
Txd = interaction among treatments and factors

Microbiological evaluation

Table 3 and Figure 4 and 5 revealed the data regarding viable count of colonies of lactic acid bacteria in terms of log CFU/ml (for free cells) and log CFU/g (for encapsulated cells). The viable colonies of each treatment were enumerated on 0, 7th, 14th, 21st and 30th day of storage. It was observed that there was a decrease in number of free lactic acid bacteria as compared to encapsulated bacteria, during storage conditions. The, maximum decrease in log CFU/ml were in Set A i.e. (10.51-10.00) and the minimum decrease was in the viable cell count was observed in Set F i.e. (10.51-10.41). Micro encapsulated probiotic ice-cream was found more stable during storage as compared to that containing free probiotic microorganisms. In addition to lactic acid bacteria, total aerobic mesophilic bacteria were also enumerated and it was observed that total aerobic bacteria were below the detection limit (<10 log CFU/ml) as shown in Table 3. Data obtained from analysis of the samples were evaluated by analysis of variance and the differences among means were calculated. Statistically, it had been confirmed that there was a significant change in viability of lactic acid bacterial free and encapsulated cells during storage and Set F contained highest number of beneficial LAB for their effective delivery to the gastrointestinal tract so as to confer the health benefits as compared to control.

Table 3 Microbial profile of probiotic free and encapsulated functional soy ice-cream

Treatments (T)	**Microbial profile (log CFU/ml) during storage period (Days)**												
	Lactic acid bacteria (Free)						**Treatments (T)**	**Lactic acid bacteria (Encapsulated)**					
	0	**7**	**14**	**21**	**30**	**Mean**		**0**	**7**	**14**	**21**	**30**	**Mean**
Set A	10.51	10.32	10.26	10.1	10.00	10.32	**Set D**	10.5	10.5	10.44	10.43	10.40	10.53
Set B	10.52	10.46	10.38	10.3	10.16	10.38	**Set E**	10.5	10.5	10.45	10.42	10.38	10.66
Set C	10.52	10.49	10.37	10.3	10.21	10.45	**Set F**	10.5	10.5	10.47	10.44	10.41	10.70
Control	2.09	2.38	2.40	2.43	2.45		**Control**	2.09	2.38	2.40	2.43	2.45	
CD0.05			A (0.10) B (0.12) Ax B (0.11)								A (0.10) B (0.12) AxB (0.27)		

Legend: Set A: *L.paracasei* S1 (free); Set B: *L..rhamnosus* KC 6 (free); Set C: *L. paracasei* S1 + *L. rhamnosus;* KC6 (free); SetD: *L. paracasei* S1(encapsulated); SetE :*L.rhamnosus;* KC6(encapsulated) Set F; *L. paracasei* S1+*L.rhamnosus* KC6(encapsulated)
CD = Critical Difference
A = Treatments
B = factors (Lactic acid bacteria free and Lactic acid bacteria encapsulated) (AxB) = Interactions among treatment and factors

Similar studies have been reported in literature, **Aboulfazli *et al.* (2016)**, prepared fermented soy ice-cream, with *Lactobacillus acidophilus* (La-05) and *Bifidobacterium bifidum* (Bb-12). The viability of probiotic microorganisms evaluated after freezing waslog10 CFU/ml and 8.2 log CFU/ml respectively. **Dertli *et al.* (2016)**, prepared ice cream fortifying *Streptococcus thermophilus* and investigates the viability of the strain and also evaluated physicochemical, rheological, molecular and sensory properties of ice cream. **Silva *et al.* (2015)**, evaluated the physicochemical characteristics, meltdown behavior and sensory properties of goat's milk ice cream, and survival of *Bifidobacterium animalis* subsp. *Lactis* BLC1, after 120 days of frozen storage, a survival rate of 84.7% was registered. **Lieu *et al.* (2017)**, evaluate the viability of *Lactobacillus casei* 431, 8.4 log CFU/ml was reported after storage period of 90 days.

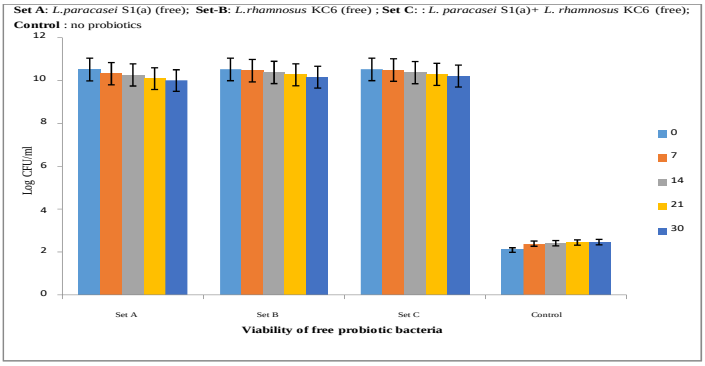

Figure 4 Survival of free probiotic bacteria in functional soy ice-cream

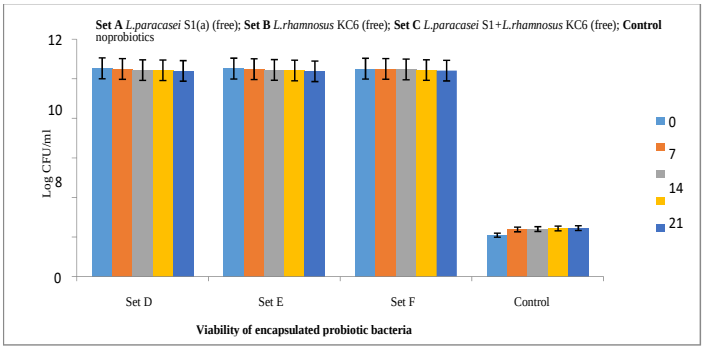

Figure 5 Survival of encapsulated probiotic bacteria in functional soy ice-cream

Captions for Figures

Figure 1: Preparation of Functional Soy Ice Cream
Figure 2: Characterization of Microencapsulated beads by Scanning Electron Microscope
Figure 3: Probiotic Enriched Soya Ice Cream
Figure 4: Survival of free probiotic bacteria in functional soy ice-cream
Figure 5: Survival of encapsulated probiotic bacteria in functional soy ice-cream

CONCLUSION

In the present investigation probiotic soya ice -cream was stored at -20^{0}C for 30 days and was investigated for microbial and physicochemical characteristics during storage days of 0,7,14,21 and 30 respectively. Microencapsulated probiotic ice cream was found more stable during storage as compared to that containing free probiotic microorganisms. As far as physico-chemical characteristics of probiotic enriched soy ice-cream is concerned, though changes in quality attributes viz. (pH, ascorbic acid, lactic acid, total soluble solid and antioxidant content) during storage interval were observed but these changes were statistically non-significant. It may be concluded that addition of health beneficial lactic acid bacteria in encapsulated form have shown high survival thus showing their high commercial utility.

Conflict of interest: There is no conflict of interest

REFERENCES

Aboulfazli, F., Bakr, S.A. & Salihin, A. (2016). Effects of the replacement of cow milk with vegetable milk on the count of probiotics and changes in sugar andamino acid contents in fermented ice creams. *Journal of Food Science and Technology*,98, 122-130. http://dx.doi.org/10.1016/j.lwt.2016.02.056
AOAC. (1995). Official methods of analysis of association of official analytica chemists, 16thedn.Vol I and II. Association of Official Analytical Chemists. Arlington,Virginia, USA. ISBN: 0-935584-42-0, ISSN: 0066-961X
Amul (2007) http://cms.businesswireindia.com
Ali, M., Alqubaisy, M., Aljaafari, M.N., et al. (2021). Nutraceuticals: Transformation of Conventional Foods into Health Promoters/Disease Preventers and Safety Considerations. *Molecules*,26(9), 2540. http://doi: 10.3390/molecules26092540
Ballini, A., Charitos, I.A., Cantore, S., Topi, S., Bottalico, L. & Santacroce, L. (2023). About Functional Foods: The Probiotics and Prebiotics State of Art. *Antibiotics,12*,635. https://dx.doi.org/10.3390/antibiotics12040635
Brower,V.(1998).Nutraceuticals: poised for a healthy slice of the healthcare market? *Nature Biotechnol*ogy 16,728-731. https://dx.doi.org/10.1038/nbt0898-728
Bajwa J S. (2018). Evaluation of probiotic potenmtial of different microbial strains isolated from food matrices and their use in formulating functional health foods. Ph.D. Thesis. Dr Y.S.Parmar University of Horticulture and Forestry Nauni, Solan. H.P. India
Cencic,A. & Chingwaru, W. (2010). Antimicrobial agents deriving from indigenous plants. *Recent Patents on Food, Nutrition & Agriculture*. 2, 83-92. https://dx.doi:org/10.2174/2212798411002010083
Dertli, E., Toker, O.S., Durak, M.Z., Yilmaz, M. T., Tatlısu, N.B.& Sagdic, O. (2016). Development of a fermented ice-cream as influenced by in-situ exopolysaccharide production: rheological molecular, microstructural and sensory characterization. *Carbohydrate Polymers*,136, 427-440. https://dx.doi.org/10.1016/j.carbpol.2015.08.04
Eratte, D., Mcknight,S.,Genhenbach, T.R. & Adhikari,B. (2015).Co-encapsulation and characterization of omega-3 fatty acids and probiotic bacteria in whey protein isolate–gum Arabic complex coacervates. *Journal of Functional Foods*, 23, 234-240. http://dx.doi./10.1016/j.jff.2015.01.037
Frakolaki, G., Giannou, V., & Tzia, C. (2022). Encapsulation of *Bifidobacterium animalis* subsp. *lactis* Through Emulsification Coupled with External Gelation for the Development of Synbiotic Systems. Probiotics and antimicrobial peptides. https:/dx./doi.org/10.1007/s12602-022-09993-7.
FAO/WHO. (2007). Probiotic in foods. Health and nutritional properties and guidelines for evaluation. *In*: FAO Food and Nutrition pp.85.Gautam, N., Sharma, N. & Ahlawat, O.P. (2014). Purification and characterization of bacteriocin produced by *Lactobacillus brevis* UN isolated from dhulliachar: a traditional food product of north East India. *Indian Journal of Microbiology*,54 (2),185-189. http://dx.doi: 10.1007/s12088-013-0427-7
Gautam, N. & Sharma, N. (2015). A study on characterization of new bacteriocin produced from a novel strain of *Lactobacillus spicheri* G2 isolated from gundruk-a fermented vegetable product of North-East India. *Journal of Food Science and Technology*, 5, 21-27. http://dx.doi:10.1007/s13197-015-1710-x
Gilliland, S.E.& Speck, M.J. (1977). Deconjugation of bile acids by intestinal *Lactobacilli. Applied and Environmental Microbiology*, 33,15–18. http://dx.doi: 10.1128/aem.33.1.15-18.1977
Gomes, A.M.P. & Malcata X.F. (1999). *Bifidobacterium* sp. and *Lactobacillus acidophilus*:Biological, Biochemical, Technological and Therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology*, 10, 139-157. https://doi.org/10.1016/S0924-2244(99)00033-3
Gulfam, A., Guo, G., Tajebe, S., Chen, L.,Liu, Q., Yuan, X., Bai, Y., & Saho, T. (2017). Characteristics of lactic acid bacteria isolates and their effect on the fermentation quality of Napier grass silage at three high temperatures, *Journal of the science of food and agriculture*, 97(6),1931-38. https://doi.org/10.1002/jsfa.7998
Indira, M., Venkateswarulu, T.C., Abraham Peele, K., Nazneen, M.B., & Krupanidhi, S. (2019). Bioactive molecules of probiotic bacteria and their mechanism of action: A review, 3 Biotech. 9(8), 306. https://doi.org/10.1007/s13205-019-1841-2
Joslyn, M.A. & Amerine, M.A. (1964). Sensory examination of wines: desert, appetizer and related flavored wines; the technology of their production. University of California, Division of Agricultural Sciences, 357-371.
Kim, S.J. et al. (2008).Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. LWT. *Food Science and Technology*, 23, 234-240. https://doi.org/10.1016/j.lwt.2007.03.025
Lieu, M.D., Dang, T.K.T. & Nguyen, T.H. (2017). Viability of microencapsulated *Lactobacillus casei* in symbiotic mayonnaise. *Food Research*, 1,234– 239. http://doi.org/10.26656/ fr.2017.6.103
Mahony, M.O. (1985). Sensory evaluation of food: statistical methods and procedures. Marcel Dekker Inc., NewYork,132.
Mahmoud, M., Abdallah, N.A.,El-Shafei, K., Tawfik, N.F. & El-Sayed, H.S. (2020). Survivability of alginate- microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. *Heliyon*, e03541. http://doi.org/10.1016/j.heliyon.2020.e03541
Rangana, S. (1995). Handbook of analysis and quality control for fruits and vegetable products. Tata McGraw Hills Publishing Company Limited. New Delhi.
Rodrigues, D. *et al*., (2011). On the viability of five probiotic strains when immobilized on various polymers. *International Journal of Dairy Technology*, 64,137-144. https://doi.org/10.1111/j.1471-0307.2010.00627.x
Salem, M.M., Fathi, F.A. & Awad, R. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences*, 14, 267-271.

Shi, L.E., Li, Z.H., Li, D.T., & Tang, Z.X. (2013). Encapsulation of probiotic *Lactobacillus bulgaricus* in alginate – Milk microspheres and evaluation of the survival in simulated gastrointestinal conditions. *Journal of Food Engineering*, 117 (1), 99-104. http://dx.doi.org/10.1016/j.jfoodeng.2013.02.012

Silva, C.E.F., Silva, I.C.C. & Abud, A.K.S. (2015). Acidulants in Tropical Fruit Pulp: Physicochemical and Sensory Changes. *In*: Guidetti R., Bodria L. & Best S., (eds). Chemical Engineering Transactions Vol. 44, The Italian Association of Chemical Engineering.

Simone, C. De. (2018). The unregulated probiotic market. *Clinical gastroenterology and hepatology*. 17 (5), 809-817. https://dx.doi.org/10.1016/j.cgh.2018.01.018

Valdes, A.M., Walter, J. Segal, E & Spector, T.D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, 361: k2179. https://doi.org/10.1136/bmj.k2179

Würth, R., Hormannsperger, G., Wilke, J., Foerst, P., Haller, D. & Kulozik, U. (2015). Protective effect of milk protein based microencapsulation on bacterial survival in simulated gastric juice versus the murine gastrointestinal system. *Journal of Functional Foods* 15, 116–125. http://dx.doi.org/10.1016/j.jff.2015.02.046

William, W.B., Cuvelier, M.E.& Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Zhou, J.S., Pillidge, C.J., Gopal, P.K. & Gill, H.S. (2005). Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *International Journal*