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Production of Synbiotic Ice Cream

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Abstract: The aim of this study was to evaluate the survivability of three probiotic strains *Lactobacillus plantarum, Lactobacillus casei* and *Bifidobaterium bifidum* in ice cream using microencapsulation by calcium alginate and whey protein concentrate. Also, the effect of different prebiotics (inulin, lactulose and Fructo-oligosaccharides) was studied. Six types of synbiotic ice cream containing free and microencapsulated *L. plantarum, L. casei* and *B. bifidum*, were manufactured using 2% Fructo-oligosaccharides. The survival of all tested strains increased with different sugars even in the presence of FOS. Also, the viability of microencapsulated strains increased more than 1 log cycle compared with free cells. The survival of *L. plantarum, L. casei* and *B. bifidum* were monitored during the storage period of 90 days. The viable cell count of *L. plantarum, L. casei* and *B. bifidum* in the Free State in ice cream was 8.344, 8.413 and 8.230 log cfu/ g at day one and the numbers were decreased to 7.841, 6.110 and 6.436 log cfu/ g after 90 days of storage respectively. Survival of the three encapsulated probiotic strains showed that the bacterial counts increased about two log cycle during the same period of storage. The viability of probiotic cells in paramount importance because to have their beneficial effect on the health, they must stay alive until they reach their site of action and be resistant to gastrointestinal environment. Further the addition of microencapsulated probiotic strains in ice cream had no significant effect on the physiological properties of ice cream and in the sensory properties.
Keywords: Microencapsulation, Probiotic bacteria, Inulin, Lactulose, Fructo oligosaccharides.

Introduction

Functional foods are those that promote health benefits beyond basic nutritional functions, when consumed in usual diet. Prebiotics and probiotics are current examples of functional food ingredients[1]**.** Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host[2]**.** *Lactobacillus* and *Bifidobacterium* are the most used as probiotic for dairy products, which are believed to have beneficial effects on human health[3]**.** The efficiency of added probiotic bacteria depends on the dose level, temperature, type of dairy foods and presence of air[4]**,** their viability must be maintained throughout the product's shelf-life and the gut environment[5]**.** The therapeutic value of probiotic bacteria normally depends on the viability of these bacteria. Therefore, 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. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of populations of bacteria in the colon[6,7,8]**.** Prebiotics might also enhance the growth and survival of probiotics in foods[9]**.** They are specific, naturally occurring carbohydrates, mainly of plant origin, and fructo-oligosaccharides (FOS) are mostly known representatives. Fructo-oligosaccharides (FOS) are a mixture of oligosaccharides, consisting of glucose linked to fructose units. Ingested FOS are poorly digested in the human small intestine but are fermented in the colon by the resident microflora[10]. Unlike other indigestible sugars, such as inulin, lactose and lactulose, which are hydrolyzed by a wide variety of gut bacteria, FOS are only fermented in vitro by a limited range of microorganisms that include most species of bifidobacteria. Indigestible compounds that are capable of selectively stimulating the growth of beneficial bacteria. Probiotics and prebiotics may be combined to form synbiotic products that will benefit consumers with health benefits [11]. There is a synergy between probiotics and prebiotics in synbiotic products. Prebiotic compounds are consumed by probiotics as a carbon or energy source in the colon. These results in an increase in the probiotic count and the reduction of pathogen microorganisms in the gut [12]. Synbiotic formulation containing food products are

used for the development of therapeutic foods. The ice-cream matrix might be a good vehicle for probiotic cultures, due to its composition, which includes milk proteins, fat and lactose, as well as other compounds[13]**.** Although dairy ice cream seems to be a good vehicle for probiotic cultures due to its composition and pH near to 6.0, the viability of these microorganisms can be affected by freezing process and oxygen toxicity. In order to overcome these problems, microencapsulation methods can be applied to increase the survival of probiotic cultures in frozen dairy products[14-16]**.** Microencapsulation has previously been reported as a technology to protect sensitive substances against the influences of adverse environments[17]**.** The term ''microencapsulation'' designates a defined technology of packing solids, liquids or gases in small capsules, which can release their contents under specific circumstances. Microencapsulation technologies are hypothesized to be a promising prospect for introducing viable probiotic bacteria in foods because the encapsulation matrix can provide a physical barrier against harsh environmental conditions such as freezing and those encountered during gastric juice passage[18- 22]. So the objectives of this study were to investigate the survivability of microencapsulated probiotic strains and production of synbiotic ice cream.

Materials and Chemicals

Skim milk powder (medium heated, fat 1.25%, moisture 4%), white sugar, whole milk (3% fat) and vanilla were obtained from local market. CMC was obtained from BDH chemicals Ltd Poole, England. Emulsifier mono and diglyceride 60% was obtained from Misr for Food Additives (MISAD), Giza, Egypt. Inulin, lactulose and fracto-oligosaccharides were obtained from Los Angeles, CA 90035.4317 USA.

Strains

Bifidobacterium bifidum was provided by Chr. Hansen's Lab. Denmark, *Lactobacillus casei* and *Lactobacillus plantarum* were provided by the Northern Regional Research Laboratory. Illinois, (NRRL) USA.

Methods

Cultivation and harvesting of lactobacilli and bifidobacterium cells

DeMan-Rogosa- Sharpe broth (MRS broth, Oxoid) was used to prepare the cell suspensions for lactobacilli. The MRS medium was inoculated with 5% active lactobacilli strains with initial count (10^7cfu/ml) and incubated at 37 ºC for 24h. Also, using MRS broth, Oxoid supplemented with 0.5% of L-cysteine solution (10%), and 1.0% of lithium chloride solution (10%) and inoculated with 5% active *Bifidobacterium bifidum* with initial count (10^7cfu/ml) and incubated at 37 ºC for 72h under anaerobic condition.

Preparation of microencapsulated cells

All glass wares and solutions used in the protocols were sterilized at 121 °C for 15 min. Alginate beads were produced according to [23]. A probiotic cell suspension was prepared by centrifuging 80 ml of 24 hour old culture at 5000 rpm 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 $CaCl_2$. The $CaCl_2$ concentration was at 0.1M and dripping was achieved with a sterile 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.

Effect of different prebiotics on the viability of probiotic strains

To study the effect of different prebiotics on the viability of probiotic strains, three different types of sterilized probiotics (inulin, lactulose and fracto-oliogosccharides) were added to MRS broth medium by 2% and inoculated by 2% of each microencapsulated and free strains and incubated at 37 °C for 48h under anaerobic condition. The viable count of lactobacilli were determined using MRS agar according to [24] and the plates were incubated at 37°C for 48h under anaerobic condition. The viable count of bifidoacterium was determined by MRS agar supplemented with 0.5% of L-cysteine solution (10 and 1.0% of lithium chloride solution (10%) and incubation at 37 °C for 72h under anaerobic conditions. The best type of sugar which increased the viability of probiotic strains used in the manufactured of synbiotic ice cream.

Preparation of synbiotic ice cream using microencapsulated strains

Ice cream was prepared using methods [25, 26]. Ice cream base mix was prepared to contain 8% fat, 12% sucrose, 2% fracto-oligosaccharides, 0.2% stabilizer, emulsifier 0.1% and the MSNF 12%. All mixes were pasteurized at 80±1°C for 30 Second, cooled to 4°C. The mixture was divided into seven portions. The first portion as control, microencapsulated strains of *B. Bifidium*, *Lb. casei* and *L. plantarum* (10^8cfu/ml) were added to the second, third and fourth portions respectively at a rate of 2%, free strains of *B. Bifidium*, *L. casei* and *L. plantarum* (10^8cfu/ml) were added to the fifth, sixth and seventh portions respectively at the rate of 2%. The mixes aged in the fridge at 4°C for 20 h., frozen and whipped in the ice cream maker (Model: BL1380) for 30 min. The ice cream was collected at an exit temperature of – 5.5°C, placed in a 1 L. plastic container, sealed, hardened for 2 h. in a freezer and stored at -20±2°C for 24 h before analysis.

Enumeration of microencapsulated and free probiotic strains

Enumeration of probiotic bacteria was achieved as described by [27, 28]. Each sample of ice cream (25 g) were diluted in 225 mL phosphate buffer (pH 7.5) and homogenized for 5 min. Ten milliliter of this dilution was used to obtain serial dilutions in physiological solution. Lactobacilli strains were counted by pour-plating 1 mL of each dilution in MRS agar and incubation at 37 °C for 72 h under anaerobic conditions. Bifidobacterium was counted by pour-plating 1 mL of each dilution in MRS agar, Oxoid supplemented with 0.5% of L-cysteine solution (10%) and 1.0% of lithium chloride solution (10%) and incubation at 37 °C for 72 h under anaerobic conditions. Samples were taken at zero, 7, 15, 22, 30 days and as well as the end of every 30 days until 90 days of storage. The entrapped strains were released from the beads was counted in ice cream as per the procedure described by [29].

Physico-Chemical Properties of synbiotic ice cream using microencapsulated strains

Overrun and melting properties

Three batches of each type of probiotic ice cream were evaluated for overrun and melting assessment [30,26]. The overrun was calculated using the equation of " %Overrun = (Vol. of ice cream – Vol. of mix used)/Vol. of mix used X 100 ." Meltdown of frozen ice cream was determined according to[31], by carefully cutting the foamed plastic cups from the ice cream samples (~50 gm), placing the samples onto wire mesh over a glass funnel fitted on conical flask, and weighing the amount of ice cream drained into the conical flask at 30°C every 15min. until the entire sample had melted.

pH values

The pH values were determined in triplicate using laboratory pH meter (HANNA, Instrument, Portogal).

Apparent viscosity of synbiotic ice cream (c.P.s)

Probiotic ice cream samples were gently stirred 5 times in clockwise direction with a plastic spoon prior to viscosity measurements. Apparent viscosity was measured at 24 ºC using a Brookfield digital viscometer (Middleboro, MA02346, USA). The sample was subjected to shear rates ranging from 5 to 100 S-1 for upward curve. Viscosity measurements were expressed as centipoises (c.P.s) and were performed in triplicate, as described by[32].

Sensory Properties of synbiotic ice cream

Samples of synbiotic ice cream after 24 h. hardening at -18 °C were evaluated by 15 specific stuff members at Dairy Dept., National Research Center, was carried out according to [33], using scale of 50 points for flavor, 40 points for body and texture, 5 points for melting property and 5 points for appearance.

Statistical analysis

The data were analyzed according to Statistical Analysis System Users Guide [34] (SAS Institute, Inc, U.S.A.). Separation among means in triplicates was carried out using Duncan multiple test.

Results and Discussion

Effect of different sugars on the viability of free and microencapsulated strains

Table (1) shows the effect of different prebiotics on survival of free and microencapsulated strains. Obviously, the survival of all tested strains increased with different prebiotics even in the presence of FOS. Also, the viability of microencapsulated strains was more than free cells strains which the viability increased more than 1 log cycle compared with free cells. Microencapsulated (*L. plantarum*, *L. casei* and *B. bifidium* increased in the presence of FOS from initial count (9.150 9.175 and 9.083 log cfu/g) to (10.336, 10.165 and10.389 log cfu/g) respectively, but the free cells of the same strains in the presence of FOS increased from (7.016, 7.196 and 8.165 log cfu/g) to (8.495, 8.411 and 8.502 log cfu/g) respectively. Fritzen-Freire *et al.*, [35] showed that the microcapsules produced with oligofructose-enriched inulin and those produced with oligofructose showed better protection for the bifidobacteria during storage. Also, Kaplan and Hutkins[36] examined 28 strains of lactic acid bacteria and bifidobacteria, 12 of 16 Lactobacillus strains and 7 of 8 *Bifidobacterium* strains fermented FOS. Only strains that gave a positive reaction by the agar method reached high cell densities in broth containing FOS. Champagne *et al.*[37] reported that immobilized systems can reach higher cell densities than classical free cell fermentation performed under the same conditions.

Table (1) Effect of different prebiotics on the viability of free and microencapsulated probiotic strains (Log cfu/g).

Strains	Initial counts	Inulin	Lactulose	FOS
L. plantarum	7.016^{Cb}±0.013	7.109^{Bb}±0.056	8.500^{Ab}±0.005	8.495^{Ab}±0.350
Cap. *L. plantarum*	9.150^{Ba}±0.040	9.353^{Ba}±0.040	9.495^{Ba}±0.010	10.336^{Aa}±0.035
L. casei	7.196^{Bb}±0.009	7.208^{Bb}±0.017	8.328^{Bb}±0.553	8.411^{Ab}±0.655
Cap. *L. casei*	9.175^{Ca}±0.004	9.294^{Ba}±0.030	9.305^{Ba}±0.004	10.165^{Aa}±0.031
B. bifidum	8.165^{Bb}±0.036	8.373^{Ab}±0.052	8.288^{Bb}±0.035	8.502^{Ab}±0.025
Cap. *B. bifidum*	9.083^{Ca}±0.011	9.838^{Ba}±0.038	10.081^{Aa}±0.020	10.389^{Aa}±0.034

Data expressed as mean of 3 replicates ±standard error. Means in the same row showing the same capital letters are not significantly different (P≤ 0.05). Means in the same column showing the same small letters are not significantly different (P≤ 0.05). Cap: Microencapsulation strains.

Viability of free and microencapsulated strains in ice cream during storage periods at freezing temperature

Survivability of three proven probiotic *L. plantarum*, *L. casei* and *B. bifidum* were enumerated at day one and at the end of 90 days of storage showed a significant difference ($p \leq 0.05$). Generally, the number of free and microencapsulated strains decreased continuously with the increased in the storage period. The viable counts presented in Tables (2, 3 and 4). Free *L. plantarum*, the cell number dropped substantially from 8.344 to 5.983 log cfu/g (about 2.4 log number) from day one to 90 days of storage, wherein microencapsulated *L. plantarum*, the cell number decreased from 9.378 to 7.970 log cfu/g (about 1.4 log number). The *L. casei* count showed an average 2.3 log reduction in free state from 8.413 to 6.110 log cfu/g during day one to 90 days, wherein microencapsulated state of the same strain showed a decreased count from 9.482 to 8.400 log cfu/g respectively (about 1.082 log number). Also, free *B. bifidum* cells count decreased from 8.230 to 6.436 log cfu /g at the end of storage (about 1.8 log number), moreover the microencapsulated cells of the same strain showed a decreased from 9.486 to 8.143 log cfu/g respectively. Microencapsulated cells survived freezing better than free cells ($P \leq 0.05$) when compared within the same strain. About two log numbers more survival rate was observed when the probiotic strains were encapsulated than when they were free. Protection by microencapsulation was significant ($P < 0.05$) in the ice cream as well as during storage period at freezing temperature. These results were agreement with Shah and Ravula [16]**.** Who reported that microencapsulation improved the counts of *Lactobacillus acidophilus* and *Bifidobacterium* spp. 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 [29]. Moreover Karthikeyan *et al.*, [23] observed that 30 per cent more survived rate when the probiotics were encapsulated in calcium alginate than when they were not encapsulated. Homayouni *et al.*, [38] Found when encapsulated *Lactobacillus casei* (Lc-01) and *Bifidobacterium lactis* (Bb-12) bacteria in calcium alginate beads the probiotic survival raised at rate of 30% during the same

period of storage at same temperature. In general, the results indicated that encapsulation can significantly increase the survival rate of probiotic bacteria in ice cream over an extended shelf-life.

Table (2) Viability of free and microencapsulated *L. plantarum* in ice cream during storage periods at freezing temperature (Log cfu/g).

Storage periods (days)	Free *L. plantarum*	Microencapsulated *L. plantarum*
Zero	8.344^{Ab}±0.027	9.378^{Aa}±0.0421
7	8.295^{Bb}±0.024	9.410^{Aa}±0.052
15	8.061^{Bb}±0.033	9.245^{ABa}±0.056
21	8.088^{Bb}±0.018	9.159^{ABa}±0.027
30	7.841^{Cb}±0.032	9.051^{Ba}±0.013
60	6.376^{Db}±0.062	8.667^{Ca}±0.142
90	5.983^{Eb}±0.103	7.970^{Da}±0.136

Data expressed as mean of 3 replicates ±standard error. Means in the same row showing the same small letters are not significantly different (P≤ 0.05). Means in the same column showing the same capital letters are not significantly different (P≤ 0.05).

Table (3) Viability of free and microencapsulated *L. casei* in ice cream during storage periods at freezing temperature (Log cfu/g).

Storage periods (days)	Free *L. casei*	Microencapsulated *L. casei*
Zero	8.413^{Ab}±0.017	9.482^{Aa}±0.029
7	8.295^{ABb}±0.032	9.371^{Aa}±0.41
15	8.112^{BCb}±0.009	9.362^{Aa}±0.047
21	7.986^{Cb}±0.015	9.173^{Ba}±0.027
30	7.870^{Cb}±0.027	9.210^{Ba}±0.054
60	6.860^{Db}±0.017	8.926^{Ca}±0.037
90	6.110^{Eb}±0.168	8.400^{Da}±0.028

Data expressed as mean of 3 replicates ±standard error. Means in the same row showing the same small letters are not significantly different (P≤ 0.05). Means in the same column showing the same capital letters are not significantly different (P≤ 0.05).

Table (4) Viability of free and microencapsulated *B. bifidum* in ice cream during storage periods at freezing temperature (Log cfu/g).

Storage periods (days)	Free *B. bifidum*	Microencapsulated *B. bifidum*
Zero	8.230^{Ab}±0.055	9.486^{Aa}±0.009
7	8.301^{Ab}±0.007	9.330^{Aa}±0.061
15	8.139^{Ab}±0.036	9.277^{Ba}±0.041
21	8.013^{ABb}±0.046	9.122^{Ca}±0.018
30	7.863^{Bb}±0.036	9.066^{Da}±0.026
60	7.393^{Cb}±0.096	8.810^{Da}±0.026
90	6.436^{Db}±0.200	8.143^{Ea}±0.082

Data expressed as mean of 3 replicates ±standard error. Means in the same row showing the same small letters are not significantly different (P≤ 0.05). Means in the same column showing the same capital letters are not significantly different (P≤ 0.05).

Physico-Chemical Properties of synbiotic ice cream using microencapsulated strains

Properties of ice cream mixes and resultant ice cream containing different probiotic free and encapsulated strains presented in Table (5). The data shows some properties of ice cream mixes, the specific gravity of all treated ice cream in the same trend ranged between 1.13 – 1.19 and specific gravity of resultant ice cream was ranged between 0.65 – 0.71. Specific gravity of all resultant ice cream mixes found to be closely significantly related to specific gravity of ice cream mixes. The data shows also to the overrun values which refer to overrun slightly significant different, the overrun ranged between 63.46 to 66.56 %. Addition of different probiotic strains in case free or microencapsulated don't affect significantly on physical properties of ice cream mixes. Also, pH value in ice milk mixes decreased slightly with the ice cream mixes containing probiotic strains and more decreased in mixes contains microencapsulated probiotic strains than the control. The increase in acidity of mix contain microencapsulated probiotic strains may be due to the effect of activity of strains increased with increasing of protection of strains by microencapsulation. The results are in agreement with Karthikeyan *et al*.,[23] who found that there is no significant difference in the chemical and physical characteristics.

Table (5) Changes in the properties of ice cream mixes and ice cream.

Treatments	Specific gravity For mixes	Specific gravity For ice cream	Overrun %	pH
Control	1.16^{BC}±0.056	0.69^{BC}±0.056	65.45^{B}±0.055	6.64^{A}±0.057
L. plantarum	1.15^{CD}±0.052	0.70^{AB}±0.057	64.10^{F}±0.047	6.49^{B}±0.056
Cap. *L. plantarum*	1.13^{E}±0.048	0.68^{C}±0.053	64.60^{E}±0.048	6.44^{D}±0.051
L. casei	1.14^{DE}±0.049	0.65^{D}±0.50	65.20^{D}±0.050	6.46^{C}±0.053
Cap. *L. casei*	1.16^{BC}±0.056	0.71^{A}±0.057	63.64^{G}±0.045	6.38^{E}±0.047
B. bifidum	1.19^{A}±0.057	0.71^{A}±0.057	66.56^{H}±0.044	6.50^{B}±0.056
Cap. *B. bifidum*	1.17^{B} ±0.055	0.69^{BC}±0.056	65.27^{C}±0.053	6.33^{F}±0.047

Data expressed as mean of 3 replicates ±standard error. Means in the same column showing the same capital letters are not significantly different ($P \leq 0.05$).

Apparent viscosity of synbiotic ice cream (c.P.s)

Apparent viscosity corresponds to the amount of force required to move one layer of fluid in relation to another in the ice cream mix. Viscosity values (c.P) are plotted as a function of time (s) in Fig. (1), to show the flow behavior of ice cream samples with different microencapsulated strains. The viscosity behavior is influenced by the complex hydrodynamic properties (i.e., size, shape, and hydration potential). The value of apparent viscosity in the control treatment (0% FOS) ice cream was found to be slight differences to the value of apparent viscosity in 2.0% FOS in the other synbiotic ice cream samples. Also, the viscosity of synbiotic ice cream significantly decreased with the increased of time (s) in all treatments. The results revealed that, adding of different free or microencapsulated probiotic strains in this study don't affect in apparent viscosity of different ice cream mixes compared with control and addition of FOS made slight differences in ice cream samples. Akalin *et al.*, [39] reported that the viscosity values did not vary between the samples of regular ice cream and reduced-fat or low-fat ice cream containing inulin or oligofructose.

Melting properties of synbiotic ice cream

Melting resistance (Loss% after) of free or microencapsulated probiotic strains founded in ice cream samples founded in Table (6). Melting resistance of ice cream was expressed as the loss in weight percent of the initial weight of the tested samples. The melting resistance of free or microencapsulated probiotic ice cream samples significantly increased with increasing the time of the test. The free and microencapsulated probiotic ice cream slightly different compared with control treatment, addition of probiotic bacteria free or microencapsulated don't affect in melting resistance of ice cream as showed in Table (6). All tested samples in this study have the same melting properties as control.

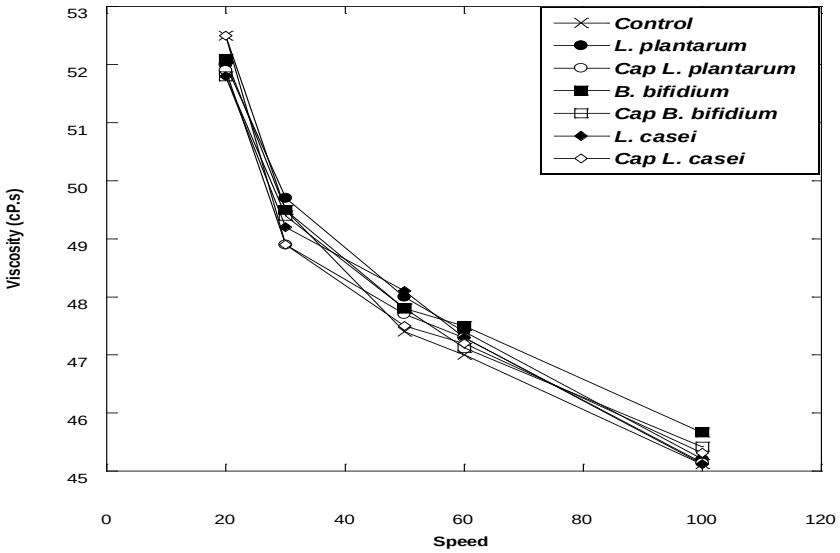

Fig. (1) Viscosity of synbiotic ice cream mixture

Table (6) Melting resistance (Loss% after) of probiotic ice cream samples

Treatments	Time (min)			
	15	**30**	**45**	**60**
Control	10.48^{De}±0.040	35.65^{Cd}±0.045	65.68^{Ba}±0.55	75.16^{Ad}±0.057
L. plantarum	11.12^{Db}±.033	35.66^{Ccd}±0.048	66.02^{Ba}±0.050	74.79^{Af}±0.056
Cap. *L. plantarum*	10.99^{Dc}±0.038	35.77^{Ca}±0.040	65.66^{Ba}±0.049	75.67^{Aa}±0.055
L. casei	11.02^{Dc}±0.033	35.38^{Ce}±0.036	65.23^{Ba}±0.040	75.26^{Ac}±0.057
Cap. *L. casei*	10.73^{Df}±0.032	35.68^{Cb}±0.039	66.10^{Ba}±.044	75.39^{Ab}±0.052
B. bifidum	10.53^{Dg}±0.030	35.22^{Cf}±0.037	65.45^{Ba}±0.042	74.98^{Ae}±0.054
Cap. *B. bifidum*	11.23^{Da}±0.034	35.67^{Cbc}±0.038	66.12^{Ba}±0.045	75.21^{Aa}±0.057

Data expressed as mean of 3 replicates ±standard error. Means in the same row showing the same capital letters are not significantly different ($P \leq 0.05$). Means in the same column showing the same small letters are not significantly different ($P \leq 0.05$).

Sensory Properties of synbiotic ice cream

Sensory analysis of symbiotic ice cream which contained free and microencapsulated strains was showed in the Table (7). Data showed that the addition of free and microencapsulated probiotics strains in ice cream had no effect on sensory properties of probiotic ice cream. The overall acceptability of free and microencapsulated probiotic ice cream samples in storage period were ranged between 87 - 86.3. The addition of microencapsulated probiotics strains in ice cream had no significant effect on the sensory properties. Overall acceptability in terms of texture and taste of all samples were good and no marked off flavor was found during the storage period. This results agreement with Karthikeyan *et al.*,[23] who found that the addition of microencapsulated probiotics in ice cream had no significant effect on the sensory properties. Also, our results near to Raj and Prasad[40] who found that no significant differences in the means values of body, appearance, color and texture score of control and samples of ice cream which contained microencapsulated *L. plantarum* throughout the storage period. the addition of encapsulated probiotics had no significant effect on the sensory properties of non-fermented ice cream in which we used the resistant starch as prebiotic compound [38].

Table (7) Sensory evaluation of probiotic ice cream samples

Properties	Treatments						
	Control	***L. plantarum***	**Cap. *L. plantarum***	***L. casei***	**Cap. *L. casei***	***B. bifidum***	**Cap. *B. bifidum***
Flavor (50)	$45.3^{B}\pm0.056$	$45.5^{A}\pm0.055$	$45.2^{BC}\pm0.052$	$45.6^{A}\pm0.057$	$45.3^{B}\pm0.055$	$44.9^{D}\pm0.046$	$45.1^{C}\pm0.046$
Body & texture (40)	$34.2^{D}\pm0.048$	$34.4^{C}\pm0.049$	$34.7^{A}\pm0.057$	$34.1^{D}\pm0.046$	$34.5^{BC}\pm0.053$	$34.6^{AB}\pm0.052$	$34.2^{D}\pm0.044$
Melting properties (5)	$3^{A}\pm0.057$	$3^{A}\pm0.058$	$3^{A}\pm0.055$	$3^{A}\pm0.056$	$3^{A}\pm0.057$	$3^{A}\pm0.056$	$3^{A}\pm0.057$
Appearance (5)	$4^{AB}\pm0.054$	$4.1^{A}\pm0.057$	$4^{AB}\pm0.054$	$4.1^{A}\pm0.056$	$4^{AB}\pm0.053$	$3.9^{B}\pm0.050$	$4^{AB}\pm0.053$
Total Score (100)	$86.5^{C}\pm0.0$	$87^{A}\pm0.056$	$86.9^{AB}\pm0.053$	$86.8^{B}\pm0.055$	$86.8^{B}\pm0.0$	$86.4^{CD}\pm0.045$	$86.3^{D}\pm0.047$

Data expressed as mean of 3 replicates ±standard error. Means in the same column showing the same capital letters are not significantly different ($P\leq 0.05$). Cap.: Microencapsulated.

Conclusions

The study indicates that probiotic survivability in ice cream can significantly improved by microencapsulation. Also, the microencapsulated of probiotic strains not have any effect on the physiological properties and sensory evaluation of synbiotic ice cream. The acceptability of the synbiotic ice cream containing microencapsulated strains was satisfactory.

References

1. Siró, I., Kápolna, E., Kápolna, B. and Lugasi, A. Functional food. Product development, marketing and consumer acceptance – a review. Appetite, 2008, 51(3): 456 – 467.
2. FAO/WHO.: *Food and Agricultural Organization: Experts Report*. Guidelines for the Evaluation of Probiotics in Food, 2001. http:// www.fao.org.
3. Saxelin M., Tynkkynen, S., Sandholm, M.T. and deVos, W.M. Probiotic and Other Functional Microbes: From Markets to Mechanisms. Current Opinion in Biotechnology. 2005, 16; 04-211.
4. Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S. and Razavi, S. H. A review on the method of increasing probiotic survival in functional dairy foods. In Proceedings of the 9th Iranian nutrition congress, 2006, (pp. 288–297).
5. Kailasapathy, K. and Chin, J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunology and Cell Biology,2000, 78: 80–88.
6. Gibson, G. R. and Roberfroid, M. B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. The Journal of Nutrition, 1995, 125 (6): 1401-1412.
7. Charalampopoulos, D., Pandiella, S.S. and Webb, C. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. International Dairy of Food Microbiology, 2003, 82 (2): 133-141.
8. Roberfroid, M. Prebiotics: The Concept Revisited. The Journal of Nutrition, 207, 137: 830S-837S.
9. Akin, M. S. Effects of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensorial characteristics of probiotic fermented ice-cream. Milchwissenschaft, 2005, 60(3), 297-300.
10. Stone-Dorshow, T. and Levitt, M.D. Gaseous response to ingestion of a poorly absorbed fructo-oligosaccharide sweetener. Am J. Clin Nutr., 1987, 46: 61–65.
11. Frost and Sullivan. European and United States Probiotic Market. 2003, *http://www.marketresearch.com/product/display.asp?product*.
12. Vernazza, C. L., Rabiu, B. A. and Gibson, G. R. Human colonic microbiology and the role of dietary intervention: Introduction to prebiotics. In G. R. Gibson & R. A. Rastall (Eds.), Prebiotics: Development and application, 2006, (pp. 1–12). England: John Wiley & Sons Ltd.
13. Cruz, A.G., Antunes, A.E.C., Sousa, A.L.O.P., Faria, J.A.F. and Saad, S.M.I. Ice cream as a probiotic food carrier. Food Research International, 2009, 42: 1233–1239.
14. Anal, K. A. and Singh, H. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Sci. and Technol., 2007, 18, 240-251.
15. Capela, P., Hay, T. K. C. and Shah, N.P. Effect of homogenization on bead size and survival of encapsulated probiotic bacteria. Food research International, 2007, 40, 1261-1269.
16. Shah, N. P. and Ravula, R. R. Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. The Australian J. Dairy Technol., 2000, 55(3): 139-144.
17. Champagne, C.P. and Fustier, P. Microencapsulation for the improved delivery of bioactive compounds into foods. Biotechnol., 2007 18: 184–190.
18. Boh, B. Developments of applications industries des microcapsules. In: Vandamme, Thierry F. (Ed.), Microencapsulation: Des Sciences Aux Technologies. Lavoisier, Paris, 2007, pp. 9–22.
19. Capela, P., Hay, T.K.C. and Shah, N.P. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. Food Res. Int., 2006, 39, 203–211.
20. Champagne, C.P. and Kailasapathy, K. In: Garti, N. (Ed.), Controlled Release Technologies for Targeted Nutrition. Wood head Publishing, CRC Press, Cambridge, UK, 2008, pp. 344–369.
21. Kailasapathy, K. Microencapsulation of probiotic bacteria: technology and potential applications. Curr. Issues Intest. Microbiol., 2002, 3, 39–48.
22. Shah, N.P. Probiotic bacteria: selective enumeration and survival in dairy foods. J. Dairy Sci., 2000, 83: 894–907.

23. Karthikeyan, N., Elango, A., Kumaresan, G., Gopalakrishnamurty, T.R. and Pandiyan, C. Augmentation of Probiotic Viability in Ice Cream Using Microencapsulation Technique. Int. J. Advanced Veterinary Sci. and Technol., 2014, 2(1): 76-83.
24. De Man, J. C., Rogosa, M. and Sharp, M. E. A medium for the cultivation of *lactobacilli*. J. Applied Bact., 1960, 23: 130-136.
25. Marshall, R. T. and Arbuckle, W. S. Ice Cream (5^{th} edition). New York: Chapman and Hall, 1996.
26. Schmidt, K. A. Dairy: Ice cream. Food processing – Principles and applications, 2004 (pp.287-296). Ames, IA: Blackwell publishing.
27. Haynes I.N. and Playne M.J. Survival of Probiotic Cultures in Low Fat Ice Cream. Australian J. Dairy Technol., 2002, 57 (1): 10-14.
28. Lourence H.A. and Viljoen B. Yogurt as Probiotic Carrier Food. Int. Dairy J., 2002, 11: 1-17.
29. Sheu, T.Y. and Marshall, R.T. Microencapsulation of lactobacilli in calcium alginate gels. J. Food Sci., 1993, 54: 557-561.
30. Arbukle, W.S. Ice cream (Fourth Edition). New York; Van Nostrand Reinhold, 1986.
31. Arndt, E.A. and Wehling, R.L. Development of hydrolyzed and hydrolyzed isomerized syrups from cheese whey ultrafiltration permeate and their utilization in ice cream. J. Food Sci., 1989, 54 (4):880-884.
32. Elling, J.L. and Duncan, S. E. Physical properties of 20 % milk fat reformulated creams manufactured from cholesterol-reduced butter oil. J. Food Sci., 1996, 61: 375.
33. Ismail, E. A. I., Al-Saleh, A. A. and Metwalli, A. A. M. Effect of Inulin Supplementation on Rheological Properties of Low-Fat Ice Cream. Life Science Journal, 2013, 10 (3): 1742-1746.
34. SAS Institute. SASA/STAT User's Guide: Statistics. Ver 6.04, Fourth Edition SAS Institute Inc., Cary, NC.,1994.
35. Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D.M.C. and Pinto, S. S. Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. Food Res. Int., 2012, 45: 306–312.
36. Kaplan, H. and Hutkins, R.W. Fermentation of Fructooligosaccharides by lactic acid bacteria and bifidobacteria. Applied and Env. Microbiol., 2000, 66 (6): 2682-2684.
37. Champagne, C.P., Cote, C. and Goulet, J. Whey fermentation by immobilized cells of *Propionibacterium shermanii*. J. Appl. Microbiol., 1989, 66: 175-184.
38. Homayouni, A., Ehsani, M. R., Azizi, A., Yarmand, M. S. and Razavi, S. H. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chemistry, 2008, 111: 50–55.
39. Akalin, A. S. and Erisir, D. "Effect of inulin and oligofractose on rheological characteristic and prebiotic culture survival in low-fat prebiotic ice cream". Inst. Food Tech., 2008, 73: 184-188.
40. Raj, A.A.P and Prassad, V. Survivability of microencapsulated *Lactobacillus acidophilus* LA-5 in low fat synbiotic ice cream. Egyptian J. Dairy Sci., 2014, 42:7-15.

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