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Effect of incorporation of inulin on the survivability of *Lactobacillus acidophilus* in synbiotic ice cream

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Abstract

A study was carried out to prepare synbiotic ice cream incorporating *Lactobacillus acidophilus* and inulin and viability of *L.acidophilus* was analyzed on storage. Whey protein concentrate (WPC) was incorporated in the ice cream mix to improve the textural and nutritional quality of ice cream. A faster melting rate was noticed in the probiotic and synbiotic ice cream samples. Incorporation of inulin in ice cream mix significantly ($P<0.01$) improved the growth of *Lactobacillus acidophilus*. Freezing of the ice cream mix caused a reduction of 0.61 to 0.77 log counts of *L. acidophilus* count. A significant reduction ($P<0.01$) in the count of *L. acidophilus* was observed during storage. It is concluded that incorporation of inulin increases count of *L. acidophilus* and the organism could survive at therapeutic minimum probiotic level of 10^6 cells /ml for 15 days of storage at -18 to -23°C in ice cream..

Introduction

Foodstuffs containing probiotics and prebiotics have been considered to be beneficial to health for many years, but only in recent years has there been scientific support for these beliefs. A functional food is a part of an everyday diet and is demonstrated to offer health benefits. The functional foods comprise: (i) conventional foods containing naturally occurring bioactive substances (e.g., dietary fiber), (ii) foods enriched with bioactive substances (e.g., probiotics, antioxidants), and (iii) synthesized food ingredients introduced to traditional foods (e.g., prebiotics). Probiotics and prebiotics may be combined to form synbiotic products that will benefit consumers more with health benefits (Frost and Sullivan, 2003). Synbiotic formulation containing food products are much used for the development of therapeutic foods. Ice cream possesses only nutritional qualities. The ice-cream matrix might be a good vehicle for probiotic cultures (Cruz *et al*., 2009). *Lactobacillus acidophilus* is a well known probiotic bacteria possesses many therapeutic properties (Nivien *et al*., 2006). Inulin, a non-digestible carbohydrate, considered as a prebiotic agent and cannot be digested except through bacterial activity. They can alter the composition of human gut flora by allowing a specific fermentation which results in a bacterial community predominated by *Bifidobacteria* (Wang and Gibson, 1993). Further, incorporation of whey protein concentrate (WPC) in ice cream further improves the textural and nutritional quality. Hence, the study was conducted to prepare synbiotic ice cream incorporating *L. acidophilus* and inulin and to assess the survival of *L. acidophilus* during storage.

Materials and Methods

Fresh cow milk was procured from the livestock farm, Veterinary College and Research Institute, Namakkal, Tamil Nadu state, India. WPC (82 per cent protein) was procured from Kanishka Flora Chem (India), Chennai and inulin were procured from Himedia Laboratories Private Limited, Mumbai, India, butter and SMP were procured from Aavin Dairy, Salem, Tamil Nadu, India, stabilizer, emulsifier, sugar, and vanilla flavour were procured from the local market in Namakkal town. Freeze dried cultures of *Lactobacillus acidophilus* NCDC 14, which has proven therapeutic benefits (Reddy et al., 2006), was obtained from National Collection of

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Table 1. Ingredients for various treatments of ice cream mix

Ingredients	Tc	T1	T2	T3
Milk	711.67	711.67	711.67	711.67
Skimmed milk powder	46	46	41.4	41.4
Whey protein concentrate	--	-	4.6	4.6
Butter	89	89	89	89
Sugar	150	150	150	147
Stabiliser and Emulsifier	3.33	3.33	3.33	3.33
Inulin (%)	-	-	-	3
Lactobacillus acidophilus (%)	-	4	4	4
Total	1000	1000	1000	1000

Table 2. Properties of various experimental ice cream samples

Parameters (n=4)	Control	T1	T2	T3
Fat %	10.08 ±0.08	10.05 ±0.03	10.10 ±0.04	10.08 ±0.08
Total solids %	36.05 ± 0.05	36.13 ± 0.03	36.08 ± 0.02	36.1 ± 0.05
Protein %(p<0.01)	4.73^{b} ±0.01	4.72^{b} ±0.01	5.08^{a} ±0.02	5.09^{a} ±0.01
Ice cream, g melted (in minutes) (p<0.01)	6.52^{a} ±0.03	6.43^{a} ±0.01	6.36^{b} ±0.03	6.32^{c} ±0.02
Coliform count (cfu/ml)	1.46 ±0.02	1.38 ±1.03	1.41 ±0.02	1.44 ±0.01
Sensory analysis (n=6)				
Flavour (10)	8.50 ±0.26	8.25 ±0.11	8.22 ±0.10	8.17 ±0.08
Body and Texture (5)	4.17 ±0.11	4.17 ±0.11	4.08 ±0.20	3.90 ±0.14
CAP (Colour, Appearance and Packaging) (5)	4.17 ±0.17	4.25 ±0.11	4.08 ±0.08	3.88 ±0.13
Melting Quality (3)	2.93 ±0.04	2.83 ±0.11	2.83 ±0.08	2.88 ±0.07
Bacterial Count (2)	2	2	2	2
Total score (25) (p<0.05)	22.00^{a} ±0.26	21.58^{a} ±0.24	21.38^{a} ±0.29	20.93^{b} ±0.20

Means bearing different superscripts between treatments differ significantly (P < 0.01)

Table 3. Survival of *L. acidophilus* in the ice cream samples during storage

Groups	Count $\log_{10}$ cfu/ml (Mean ± SE)			
	Ice cream mix	Ice cream during storage		
		0 day	7 day	15 day
T1	8.47^{b} ±0.02	7.75^{b}±0.09	7.28^{b}±0.05	7.05^{b} ±0.05
T2	8.51^{b} ±0.04	7.90^{b} ±0.14	7.33^{b}±0.12	7.11^{b} ±0.04
T3	9.60^{a} ±0.08	8.83^{a}±0.08	8.53^{a}±0.17	8.38^{a} ±0.12

Means bearing (n=4) different superscripts between treatments differ significantly (P<0.01)

Dairy Cultures, National Dairy Research Institute, Karnal, Haryana, India. The various treatment groups are presented in Table 1.

Preparation of ice cream samples

Calculation was made so as to have the level of fat (10%) and total solids (36%) in ice cream to be in compliance with the standards (Table 1) prescribed by Bureau of Indian Standards (1964). Accordingly, the required quantity of milk (Table 1) and butter was heated to 65°C and then homogenized by a two stage homogenizer (I stage 2500 psi and II stage 500 psi) to make uniform emulsion (Arbuckle, 1972). The mix was then heated to 75°C, followed by addition of the skimmed milk powder, sugar, inulin, stabilizer and emulsifier with constant stirring so as to dissolve the constituents completely. The inclusion level of inulin (3 per cent) was selected based on the pilot study. Inulin added at the level of 3 per cent was found to be optimum for therapeutic benefits (Kurien *et al*., 2005). The mix was pasteurized at 80°C for 30 minutes and aged overnight at 5°C. After ageing the ice cream mix was heat treated to a temperature of 80°C for 30 sec and cooled to 40°C and the *L. acidophilus* was inoculated into ice cream mix at the rate of 4 per cent and incubated at 40°C until the pH of 5.5 is reached. The pH of ice cream samples were recorded using (Eutech and Oakton instruments, Malaysia) digital pH meter. The ice cream samples with of pH of 4.5, 5.0 and 5.5 were prepared and the pH 5.5 was acceptable by the sensory panel (Hekmat and McMahon, 1992). The ice cream mix for the each treatment was transferred to a batch freezer and freezing was carried out separately. After freezing, the ice cream was filled in 50 ml food grade paper cups, covered with food grade lids and stored at -18°C to -23°C.

Chemical and microbiological properties

The fat, total solids, protein content and coliform count of ice cream samples were analyzed as per the procedure described in Bureau of Indian Standards (1964). The melting quality of ice cream was determined by placing a spoon full of ice cream sample on a sieve; exact time taken for the first drop of molten ice cream to fall through the sieve was recorded at room temperature (Khillari *et al*., 2007). The count of *L. acidophilus* in the ice cream mix and ice cream was evaluated (Day 0, 7 and 15) using Reinforced clostridial agar with bromocresol green and clindamycin (RCBC) by serial dilution technique (Darukaradhya *et al*., 2005).

Sensory evaluation

The control and experimental ice cream samples were subjected to sensory evaluation using modified version of ADSA ice cream score card by a panel of six judges (Bodyfelt *et al*., 1988). Maximum scores (Table 2) allotted for flavour, body and texture, melting quality, colour, appearance and packaging (CAP) and bacterial count were 10, 5, 3, 5 and 2 respectively. Statistical analysis of data of the four replications for various analyses was carried out by using completely randomized block design (Snedecor and Cochran, 1989).

Results and Discussion

The mean fat and total solids percentage of the control and treatments were maintained at 10 and 36 per cent levels as prescribed by the Bureau of Indian Standards (1964). It is inferred from the Table 2, that the statistical analysis of the data for fat and total solids showed no significant difference between control and treatments. A significant ($P<0.01$) increase in protein content (Table 2) in the treatments T2 and T3 can be attributed to the use of whey protein concentrate. The use of WPC would allow the maintenance of high protein levels with consequent nutritional and possible functional benefits. Addition of WPC to frozen desserts not only increases the protein content of the ice cream but also improves sensory and textural qualities (Antunes *et al*., 2005; Pardo *et al*., 2009). The melting quality of ice cream samples containing whey protein concentrate significantly ($P<0.01$) increased with the increasing level of WPC. A faster melting rate was observed in the probiotic and synbiotic ice cream samples. The differences in the melting behaviour of ice cream samples added with probiotic cultures may be attributed to the differences in freezing points and viscosity (Akalin and Erisir, 2008; Trindade *et a*l., 2007). Our results are in agreement with the findings of Antunes *et al*., (2005) who found that addition of WPC to frozen desserts not only increases the protein content of the ice cream but also improves sensory and textural qualities. The probiotic and synbiotic ice cream samples showed no significant difference in the coliform count ($\log_{10}$cfu/ml) as compared to control (Table 2) and were within the limit as prescribed by the Bureau Indian Standards (1964).

The flavour, body and texture, CAP and melting quality scores for the control and treatments showed no significant difference. There was a significant difference ($P<0.05$) in the total sensory score of control and treatments (Table 2) and the treatments received a lower value than the control. Supplementation of *L. acidophilus* was found to have a minimal effect on the total sensory score of the ice cream due to its higher buffering capacity as supported by Hekmat and McMahon, (1992); Salem *et al*., (2005).

The *L. acidophilus* count ($\log_{10}$ cfu/ml) of control and treatment ice cream mixes (Table 3) showed a ($P<0.01$) significant difference. The growth of *L. acidophilus* was better in ice cream mix supplemented with inulin (T3). This might be due to the growth promoting effect of prebiotics. Our findings correlate with the reports of Akin *et al*., (2007); Akin (2005).

There was a significant ($P<0.01$) difference in the count of *L. acidophilus* between different treatments after freezing. The count of L. acidophilus during initial freezing of the ice cream caused a reduction of 0.61 to 0.77 log counts and the decline in the bacterial numbers, due to freezing step is most likely due to death of sensitive cells. However, the mechanical stresses of the mixing, freezing process and incorporation of oxygen in to the mix resulted further decrease in the bacterial count. Our results closely concur with those of Hekmat and McMahon (1992); Hagen and Narvhus (1999); Salem *et al*., (2005). Hagen and Narvhus (1999) also found that the numbers of probiotic bacteria decreased by 0.7 to 0.8 log unit in ice cream during freezing or shortly afterwards. The *L. acidophilus* count on day 7 and 15 of storage also showed a ($P<0.01$) significant difference between T1, T2 and T3 respectively. Our results are in agreement with the findings of Inoue (1998); Salem *et al*., (2005). Our results can be well supported by the findings of Haroldo *et al*., (2007) who found that probiotic ice cream prepared with 4 per cent *L. acidophilus*, stored at −25°C for 60 days had a survival rate of 87 per cent. Presence of large number of lactic acid bacteria and lactic acid might have contributed to the higher survival rate during freezing (Inoue, 1998).

Conclusion

Probiotic and synbiotic ice cream could be prepared by incorporating *L. acidophilus* and inulin to ferment ice cream mix. The ice cream samples with *L. acidophilus* were found to exert a little effect on the total sensory characteristics of ice cream. Incorporation of inulin increases the growth of *L. acidophilus* and it could survive at a therapeutic minimum probiotic level of 10^6 cells /ml for 15 days of storage at -18 to -23°C in ice cream. Therefore, ice cream could be used as a good source for delivering these probiotic bacteria to the consumers.

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