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Development of synbiotic ice cream incorporating *Lactobacillus acidophilus* and *Saccharomyces boulardii*

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Abstract: A study was carried out to prepare ice cream by incorporating *Lactobacillus acidophilus* and *Saccharomyces boulardii* as probiotics and fructo-oligosaccharides (FOS) as a prebiotic and enriched with whey protein concentrate and analyzed for the viability of probiotics during storage of ice cream and the effect FOS on survival of probiotics in human gut. The protein content and melting quality of the ice cream samples showed a significant ($P<0.01$) difference. The FOS in the ice cream mix significantly ($P<0.01$) improved the growth of probiotic cultures. A significant reduction in the count of *L. acidophilus* and *S. boulardii* was observed during storage of ice cream. Consumption of synbiotic ice cream significantly increased faecal *L. acidophilus* and *S. boulardii* counts in human volunteers. The *L. acidophilus* and *S. boulardii* count were higher in the treatments when *L. acidophilus* and *S. boulardii* were incorporated in combination than alone. On withdrawal of the synbiotic ice cream feeding, faecal samples showed lower *Lactobacillus* count and *S. boulardii* disappeared in about 7 days. It is concluded that ice cream containing FOS is an excellent medium to deliver *L. acidophilus* and *S. boulardii,* for overall improvement and maintenance of microbiota in the human gastrointestinal tract.

Keywords: Synbiotic ice cream, Lactobacillus acidophilus, Saccharomyces boulardii, fructo-oligosaccharides, gut health

Introduction

Dairy foods serve as an ideal system for delivery of probiotic bacteria to the human gastrointestinal tract due to provision of a favourable environment that promotes the growth and enhances the viability of these microorganisms (Darukaradhya *et al*., 2005; Salem *et al*., 2005). Probiotics and prebiotics may be combined to form synbiotic products that will benefit consumers with health benefits (Frost and Sullivan, 2003). 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 (Cruz *et al*., 2009). Under these circumstances, to derive maximum therapeutic effects along with its nutritional benefits, incorporation of synbiotics along with WPC into ice cream and study their effects on the intestinal microflora is of prime importance to the dairy industry. *Lactobacillus acidophilus* strains are widely used as probiotic cultures in dairy products because this species possess therapeutic properties. *Saccharomyces boulardii* is useful as bio-therapeutic agent and is being recognized to have probiotic effectiveness when used alone and / or in combination with other probiotics to support digestion (Nivien *et al*., 2006). Further, substitution of skim milk powder (SMP) with whey protein concentrate (WPC) in ice cream improves the textural and nutritional quality. Hence, the study was carried out to produce synbiotic ice cream by incorporating *Lactobacillus acidophilus, Saccharomyces boulardii* and fructo-oligosaccharides (FOS) and enriched with whey protein.

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) and FOS were procured from Kanishka Flora Chem (India), Chennai, India, butter and SMP were procured from Aavin Dairy, Salem, Tamil Nadu, India, stabilizer , emulsifier, sugar, and vanilla flavour

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Table 1. Quantity of ingredients for 1000 gram of Ice cream mix

Ingredients	Control	T1	T2	T3	T4	T5	T6	T7
Milk (g)	711.67	711.67	711.67	711.67	711.67	711.67	711.67	711.67
SMP (g)	46	46	41.4	41.4	41.4	41.4	41.4	41.4
WPC (g)	0	0	4.6	4.6	4.6	4.6	4.6	4.6
Butter (g)	89	89	89	89	89	89	89	89
Sugar (g)	150	150	150	150	150	150	150	150
S&E (g)	3.33	3.33	3.33	3.33	3.33	3.33	3.33	3.33
FOS (%)	0	-	-	-	3(30g)	3(30g)	4(40g)	5(50g)
L acidophilus (%)	-	4 %	-	4%	-	4%		4%
S. boulardii (%)	-		4%	4%			4%	4%
Total	1000	1000	1000	1000	1000	1000	1000	1000

Note: Addition of FOS and *L. acidophilus* and *S. boulardii* was not taken in the figuring of ice cream mix.

were procured from the local market in Namakkal town. Freeze dried cultures of *Lactobacillus acidophilus* NCDC 14, which has proven therapeutic benefits (Reddy *et a*l., 2006), was obtained from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, Haryana, India and the pure cultures of Saccharomyces boulardii was obtained from Dr. Reddy's Laboratories Ltd, India. The various treatment groups are presented in Table 1. Among the treatments T1,T2 and T3 were of probiotic ice cream without FOS and T5,T6 and T7 were of synbiotic in nature with added FOS.

Preparation Ice Cream Samples

Necessary calculations were made so as to have the level of fat (10%) and total solids (36%) to be in compliance with the standards prescribed by the Bureau of Indian Standards,(BIS,1964). Accordingly, calculated quantity of milk 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. The mix was then heated to 75°C, followed by addition of the skimmed milk powder, sugar, stabilizer and emulsifier with constant stirring so as to dissolve the constituents completely, then pasteurized at 80°C for 30 minutes and aged overnight at 5°C. After ageing, control ice cream mix was frozen using a batch freezer and hardened and stored at -23 to -18°C (Arbuckle, 1972). After ageing the ice cream mix for various treatments was heat treated to a temperature of 80°C for 30 sec and cooled to 37°C and the active probiotic cultures were inoculated into ice cream mix at the rate of 4 per cent and incubated at 37°C until the pH of 5.5 is reached. The pH of ice cream samples was 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. 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.

The control and experimental ice cream samples were subjected to sensory evaluation by a panel of six judges using modified version of ADSA ice cream score card as described by Bodyfelt *et al.* (1988). Statistical analysis of data of the four replications was carried out by using completely randomized design.

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 melted ice cream to fall through the sieve was recorded at room temperature (Khillari *et al.*, 2007; Muse and Hartel, 2004). The coliform count was made by using violet red bile agar as per Indian Standard (1964). The concentration 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). The concentration of *Saccharomyces boulardii* in the ice cream mix and ice cream was evaluated (Day 0, 7 and 15) using chloramphenicol yeast glucose agar by serial dilution technique.

For each treatment six healthy volunteers between the age group of 24 to 27 with uniform food habits were recruited to take part in the study. The volunteers were free from gastrointestinal complaints, were not taking any medication associated with gastro intestinal activity, had not taken any antibiotic in the preceding two weeks prior to starting the study and the food did not contain any prebiotics or probiotics or preservatives as part of their daily diet. Up on presentation the volunteers were given a study number. Exactly hundred ml of ice cream samples were served to the volunteers for 15 days. The pH of the intestinal contents was recorded using digital pH meter immediately after collection. The faecal counts of *L. acidophilus* and *S. boulardii* were made on day 0 pretreatment and on days 7 and 15 of treatment, on day 16 post treatment and finally on day 21 (7 day after withdrawal).

The volunteers were advised to collect faecal sample in a sterile plastic container. One gram of faecal sample was serially diluted up to a dilution of 109 using normal saline. One milliliter of faecal slurry from the dilutions 105 and 106 each were plated for enumeration of *L. acidophilus* using RCBC agar and *Saccharomyces* species on Sabouraud agar plates.

Sabouraud agar plate set A contained cyclohexamide (0.5 mg /litre) and gentamicin (80 mg/ litre) and set B contained gentamicin (80 mg/ litre). Set B supports the unselective growth of various yeast species, including *S. boulardii*. Set A is selective for interfering Candida species. Colonies were counted after 48 hours of incubation at 30˚C. *S. boulardii* colonies were quantified as the difference in the colony counts between the two plates (Toothaker and Elmer, 1984). The bacterial colony counts were made according to the colonial morphology and expressed total log count per gram of sample (Darukaradhya *et al*., 2005).

Results and Discussion

The mean flavour score showed a significant (P<0.01) difference between the control and treatments and no significant difference was observed in body and texture, CAP and melting quality scores. Finally, a significant difference (P<0.01) in the total score of control and treatments (Table 2) was noticed. All the probiotic and synbiotic ice cream samples scored slightly lower values in flavour scores and total scores than the control and T4. Incorporation of either *L. acidophilus* or *S. boulardii* alone and in combination, was found to exert a little effect on the flavour characteristics of the ice cream due to its higher buffering capacity as supported by Hekmat and McMahon, (1992) and Salem *et al.* (2005).

The increase in the protein content in the respective treatments is due to the addition of WPC (Table 2). The present findings are in agreement with the findings Antunes *et al*. (2005) who observed that addition of WPC to frozen desserts not only increases the protein content but also improves the sensory and textural qualities (Pardo *et al*., 2009). The melting quality of the ice cream samples (Table 2) showed a (P<0.01) significant difference as compared to control and T4 respectively. A faster melting rate was noticed in the probiotic and synbiotic ice cream samples. The differences in the melting behavior of ice cream samples added with probiotic cultures may be attributed to the differences in freezing point and viscosity. The same to what has been reported in previous studies (Salem *et al*., 2005; Trindade *et al*., 2007; Akalin and Erisir, 2008). The probiotic and synbiotic ice cream samples showed a (P<0.01) significant decrease in the coliform count (log_{10} cfu/ml) as compared to control and T4.

The *L. acidophilus* count (log_{10} cfu/ml) of control and treatment ice cream mixes (Table 3) showed a (P<0.01) significant difference. Growth of *L. acidophilus* was higher in treatments containing *L.*

Table 2. Properties of various experimental ice cream samples

Parameters(n=4)	Control	T1	T2	T3	T4	T5	T6	T7
Fat %	10.08	10.05	10.03	10.1	10.05	10.13	10.1	10.05
	±0.08	±0.03	±0.03	±0.04	±0.07	±0.05	±0.04	±0.05
Total solids %	36.05	36.13	36.13	36.18	36.13	36.13	36.05	36.05
	± 0.05	± 0.03	± 0.03	± 0.03	± 0.05	± 0.05	± 0.05	± 0.03
Protein %	4.73^{a}	4.72^{a}	4.73^{a}	4.76^{a}	5.10^{b}	5.13^{b}	5.12^{b}	5.13^{b}
	±0.01	±0.01	±0.01	±0.01	±0.04	±0.03	±0.02	±0.01
Ice cream, g melted	7.10^{a}	6.42^{b}	6.40^{b}	6.30^{b}	7.13^{a}	6.38^{b}	6.38^{b}	6.32^{b}
(in minutes)	±0.06	±0.05	±0.06	±0.06	±0.01	±0.03	±0.02	±0.04
Coliform	1.52^{a}	1.45^{ab}	1.40^{ab}	1.37^{b}	1.47^{a}	1.37^{b}	1.36^{b}	1.35^{b}
count (cfu/ml)	±1.52	±1.45	±1.40	±1.37	±1.47	±1.37	±1.36	±1.35
Sensory analysis (n=6)								
Flavour (10)	8.80^{a}	8.25^{b}	8.25^{b}	8.17^{b}	8.68^{a}	8.22^{b}	8.23^{b}	8.15^{b}
	±0.08	±0.11	±0.11	±0.11	±0.03	±0.06	±0.04	±0.06
Body and Texture (5)	4.17	4.17	4.17	3.92	4.30	4.13	4.10	4.08
	±0.11	±0.11	±0.11	±0.08	±0.09	±0.09	±0.09	±0.08
CAP (5)	4.18	4.07	4.10	4.08	4.08	4.08	4.05	4.05
	±0.05	±0.03	±0.10	±0.10	±0.04	±0.06	±0.06	±0.06
Melting Quality (3)	2.93	2.83	2.83	2.88	2.90	2.82	2.83	2.87
	±0.04	±0.11	±0.08	±0.07	±0.05	±0.05	±0.03	±0.03
Bacterial Count (2)	2	2	2	2	2	2	2	2
Total score (25)	22.08^{a}	21.32^{b}	21.35^{b}	21.05^{b}	21.97^{a}	21.25^{b}	21.22^{b}	21.15^{b}
	±0.19	±0.22	±0.17	±0.17	±0.14	±0.10	±0.08	±0.14

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

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

Groups	Count log_{10} cfu/ml and Mean ± SE			
	Ice cream mix	Ice cream during storage		
		0 day	7 day	15 day
T1	8.43^{b} ±0.04	7.75^{b} ±0.09	7.28^{b} ±0.05	7.05^{b} ±0.05
T3*	8.57^{b} ±0.02	8.11^{b} ±0.05	7.78^{b}±0.09	7.55^{b} ±0.09
T5	9.38^{a} ±0.08	8.89^{a} ±0.13	8.45^{a}±0.04	8.25^{a} ±0.03
T7*	9.45^{a} ±0.10	8.95^{a} ±0.09	8.55^{a} ±0.09	8.33^{a}±0.07

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

acidophilus and *S. boulardii,* which might be due to associate action and presence of WPC and FOS. Akalin and Erisir (2008) found that the survival of *L. acidophilus* La-5 and B. animalis Bb-12 were significantly enhanced with oilgofructose. The present results could be correlated with Hagen and Narvhus (1999) who observed that the initial freezing followed by hardening of ice cream at -18°C to -23°C caused a reduction of less than one log cycle in the *L. acidophilus* count. The numbers of probiotic bacteria decreased by 0.7 to 0.8 log unit in ice cream during freezing or shortly afterwards (Hekmat and McMahon, 1992). Frozen storage of probiotic ice cream prepared with *L. acidophilus* up to 12 weeks, the count decreased by 2.23 log cfu/g. Decline in the bacterial numbers, due to freezing step is most likely due to the actual freezing of all the cells, resulting in death of some cells. Mechanical stress of the mixing, freezing process and incorporation of oxygen in to the mix would have resulted further decrease in the bacterial count (Salem *et al.,* 2005).

All probiotic ice cream samples, the count of *L. acidophilus* remained above the recommended therapeutic minimum limit of 106 cfu/g, up to15 days of storage at -18°C to -23°C. Haroldo *et al*. (2007)

also 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.

The *S. boulardii* count (log_{10}cfu/ml) of control and treatment ice cream mixes (Table 4) showed a (P<0.01) significant difference. Growth of *S. boulardii* was higher in the treatments containing *L. acidophilus* and *S. boulardii* that might be due to associative action. The added WPC and FOS might also improve the growth of *S. boulardii* in the ice cream mixes and the findings are in accordance with that of Kailasapathy and Supriadi (1996) who observed that the WPC improved the culture viability due to its protein and phosphate content. The growth of S. boulardii was better in ice cream mix supplemented with oligofructose (Hattingh and Viljoen, 2001). Further these results could be correlated with Mitterdorfer *et al*, (2001) who found that *S. boulardii* can be combined with FOS in synbiotic formulations and opined that not only the probiotic yeast will be stimulated but also, the resident positive bacterial microflora of the colon. The count of *S. boulardii* (log_{10} cfu/ml) in the ice cream immediately after freezing showed a significant (P<0.01) difference between treatments. The reduction in the count of *S. boulardii* after freezing of the ice cream ranged from 0.13 to 0.21 log counts. The decline in the yeast counts, as a result of freezing, is likely due to the freeze injury, leading to the death of cells. Pardo *et al*. (2009) who demonstrated that exposure of the yeast to media of reduced aw and /or freezing / thawing process negatively affected cell vitality and pre-treatment of *S. boulardii* in a media with water activity (a_w) of 0.98 increased the survival of yeast cells to freezing and storage at -20°C for two months by 10 fold. In UHT milk, the cell population of *S. boulardii* was 8.15 to 8.5 log_{10} cfu/ml over the storage period of 29 days at 5°C (Hattingh and Viljoen, 2001). In the present study, the count ranged from 6.67 to 6.90 log_{10} cfu/ml and is in agreement with Hattingh and Viljoen (2001). All the ice cream samples stored at -18°C to -23°C for 15 days, the viable count of *S. boulardii* remained above the recommended therapeutic minimum limit. In the current study *L. acidophilus* and *S. boulardii* survived better in combination than alone in the ice cream for 21 days at -18 to 23°C without losing the probiotic level of 106 cells /ml. WPC and FOS have considerable influence on the survival of *L. acidophilus* and *S. boulardii* in the ice cream. Therefore, in the preparation of synbiotic ice cream by using *S. boulardii* along with *L. acidophilus* appears to promote beneficial effects for the consumers.

Upon feeding of ice cream samples to the human volunteers, the faecal pH on day 0 had no significant difference (Table 5) and had a significant difference (P<0.01) on day 7 and 15 respectively. Shioiri *et al.* (2006) found that the pH of human faeces after one week of ingestion of synbiotic fermented milk (FOS and L. casei) was significantly (P<0.01) lower and daily consumption of fermented milk (80 ml) containing *Lactobacillus* (1010) and FOS (2.5 g) had significantly reduced the stool pH, one week after consumption. Casiragi *et al.* (2007) found that the synbiotic milk containing *L. acidophilus* (107 cfu/ml),

Table 4. Survival of *S. boulardii* in the ice cream samples during storage

Groups	Count log_{10} cfu/ml and Mean ± SE			
		Ice cream during storage		
	Ice cream mix	0 day	7 day	15 day
T2	6.80^{b}±0.02	6.67^{b} ±0.01	6.48^{b} ±0.04	6.37^{b} ±0.02
T3*	6.86^{b}±0.03	6.72^{b} ±0.05	6.56^{b} ±0.05	6.49^{b} ±0.05
T6	7.08^{a}±0.09	6.90^{a} ±0.04	6.75^{a} ±0.02	6.64^{a} ±0.01
T7*	7.26^{a}±0.03	7.05^{a} ±0.06	6.82^{a} ±0.05	6.72^{a} ±0.03

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

Table 5. pH of faecal samples collected from human volunteers fed with experimental ice cream

Day	Tc	T1	T2	T3	T4	T5	T6	T7
0	6.64 ±0.02	6.77 ±0.06	6.78 ±0.03	6.77 ±0.05	6.62 ±0.04	6.79 ±0.05	6.78 ±0.04	6.82 ± 0.08
7	6.61^{a} ±0.04	5.80^{c} ±0.02	5.92^{b} ±0.03	5.72^{c} ±0.03	6.58^{a} ±0.04	5.65^{cd} ±0.01	5.87^{b} ±0.03	5.62^{cd} ±0.02
15	6.65^{a} ±0.03	5.59^{c} ±0.02	5.76^{b} ±0.03	5.49^{d} ±0.03	6.56^{a} ±0.02	5.44^{d} ±0.01	5.69^{b} ±0.03	5.38^{de} ±0.03
21	6.56 ±0.05	6.49 ±0.12	6.57 ±0.02	6.42 ±0.12	6.37 ±0.06	6.29 ±0.05	6.47 ±0.06	6.32 ±0.07

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

Table 6. *Lactobacillus acidophilus* count of faecal samples collected from human volunteers fed with experimental ice cream

Day	Tc	T1	T2	T3	T4	T5	T6	T7
0	5.61 ±0.03	5.65 0.01	5.61 ±0.03	5.65 ±0.02	5.62 ±0.03	5.64 ±0.02	5.64 ±0.02	5.61 ±0.02
7	5.63^{d} ±0.03	6.64^{b} ±0.01	6.47^{c} ±0.03	6.74^{a} ±0.02	5.61^{d} ±0.03	6.75^{a} ±0.02	6.59^{b} ±0.02	6.83^{a} ±0.02
15	5.63^{c} ±0.04	6.69^{b} ±0.04	6.50^{b} ±0.02	6.81^{ab} ±0.03	5.71^{c} ±0.20	7.20^{a} ±0.14	6.61^{b} ±0.02	7.35^{a} ±0.18
21	5.67^{c} ±0.06	6.61^{ab} ±0.06	6.62^{ab} ±0.06	6.72^{a} ±0.04	5.64^{c} ±0.03	6.74^{a} ±0.03	6.64^{a} ±0.06	6.82^{a} ±0.06

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

Table 7. *Saccharomyces boulardii* count of faecal samples collected from human volunteers fed with experimental ice cream

Day	Treatments			
	T2	T3	T6	T7
7	5.96^{ab}±0.17	6.18^{ab}±0.07	6.40^{a}±0.12	6.82^{a}±0.09
15	6.61^{ab}±0.05	6.72^{ab}±0.10	6.80^{a}±0.12	7.10^{a}±0.05

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

B. lactis (107 cfu/ml) and 2 per cent inulin to reduce the pH of the stool samples in human volunteers. The reduction in the pH of the faeces may be attributed to the production of short chain fatty acids by the colonic microbiota and probiotic bacteria by utilizing the prebiotic substances in the intestine (Casiragi *et al.*, 2007; Shioiri *et al*., 2006). The mean pH of the faecal samples collected from the human volunteers on day 21 for the control and treatments showed no significant difference.

L. acidophilus count ($\log_{10}$ cfu/g) of the faecal samples collected from different groups of the human volunteers on 0 day did not differ significantly (Table 4) and a significant (P<0.01) difference was observed on day 7 and 15 respectively. Combination of *S. boulardii* and *L. acidophilus* produced a significant increase (P<0.01) in the faecal *L. acidophilus* count than feeding of ice cream samples either with *S. boulardii* or *L. acidophilus*. This may be attributed to the utilization of FOS by *S. boulardii* and *L. acidophilus*. The *L. acidophilus* count ($\log_{10}$ cfu/g) on day 21 had a significant (P<0.01) difference between control and treatment groups. The present findings can be corroborated with Gibson and Roberfroid (1995) who evaluated the *S. boulardii* and FOS combination on the resident native bacterial microflora of the colon in human being and found that *S. boulardii* and FOS combination, not only stimulated the probiotic yeast but also, the resident positive bacterial flora of the colon (Costalos *et al*., 2003; Mitterdorfer *et al*., 2001). The treatments with *L. acidophilus* and *S. boulardii* combination had a higher count of *L. acidophilus* that might be due to establishment of a symbiotic relationship in the human gastrointestinal system. These findings are in agreement with Subramanian and Shankar (1983), who found that combination of *L. acidophilus, Kluyveromyces marxianus* and *Candida pseudotropicalis* in milk and found that the growth of *L. acidophilus* was stimulated in the presence of the yeasts. *Lactobacilli* appear to enhance the beneficial effect of *S. boulardii* on intestinal mucosa by releasing polyamines, which help in repairing intestinal mucosa and growth of colonic mucosa (Buts *et al*., 1994).

Our findings are in agreement with Elmer *et al.* (1999) who noticed that *S. boulardii* does not permanently colonize the gastrointestinal tract of either animals or human and is eliminated within 24 -72 hours if not reinoculated. *S. boulardii* recovery in the stools after chronic administration is known to reach a plateau by the third week and to disappear five day after treatment is discontinued (Schneider *et al*., 2005). The present study well indicates that consumption of probiotics either *L. acidophilus* or *S. boulardii* along with WPC and FOS increased the faecal *Lactobacillus* count as compared to control group. Similarly, consumption of ice cream prepared with *L. acidophilus*, and *S. boulardii* combination increased the *Lactobacillus* count in faecal sample compared to control groups. Hence, FOS can be incorporated to improve the growth and survival of probiotic organism in the gastrointestinal tract in human being.

The mean faecal *S. boulardii* count on day 0 and 21 days were found to be nil. Because *S. boulardii*, is not a common inhabitant of the gut and does not permanently colonize the gastrointestinal tract of human being. *S. boulardii* disappeared in the faeces within five days of discontinuation of oral intake (Blehaut *et al.*, 1989). The mean faecal *S. boulardii* count ($\log_{10}$ cfu/g) on day 7 and 15 had a significant difference (P<0.01) among the treatments (Table 7). Klein *et al*. (1993) found that as the *S. boulardii* dose increased, the mean steady-state concentration of *S. boulardii* also increased significantly and the percentage of recovery was dose dependant in the human volunteers. *S. boulardii* has been shown to reach steady state level in human volunteers in stool sample at 108 cfu/g, after three days oral administration with 0.5 g/day containing 1010 cfu/g twice a day (Elmer *et al.*, 1999).

Conclusion

The population of *L. acidophilus* and *S. boulardii* in the synbiotic and probiotic ice cream were above the therapeutic level during processing and storage. It is documented that consumption of synbiotic and probiotic ice cream could significantly increase the gut flora and thereby improve the health of consumers. It is also found that, combination of *L. acidophilus* and *S. boulardii* rather than either alone had a much pronounced effect on the gut of human volunteers by increasing the count. Hence, it is concluded that ice cream can effectively be used as a medium to deliver probiotic bacteria and yeast as

well as prebiotic substance like FOS to enhance the gut health of human beings.

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