International Food Research Journal 19(2): 441-446 (2012)

In vivo and *in vitro* effect of *Lactobacillus acidophilus* in synbiotic ice cream enriched with whey protein concentrate

[1*]Pandiyan,C., [2]Annal Villi, R., [1]Kumaresan,G., [3]Murugan,B. and [4]Gopalakrishnamurthy, T.R.

[1]*Department of Dairy Science, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal sciences University, Namakkal – 637002, Tamil Nadu state, India*
[2]*Associate Professor and Head, Veterinary University Training and Research Centre, Tamil Nadu Veterinary and Animal sciences University, Vellore - 632009, Tamil Nadu, India*
[3]*Associate Professor, Livestock Research Station, Tamil Nadu Veterinary and Animal sciences University, Kattupakkam, Chenglepet - 603203, Tamil Nadu state, India*
[4]*Associate Professor, Avian Diseases Laboratory, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal sciences University, Namakkal – 637002, Tamil Nadu state, India*

Abstract: A study was conducted to prepare synbiotic ice cream by incorporating prebiotic substances viz., honey, fructo-oligosaccharides and inulin along with *Lactobacillus acidophilus* in ice cream, to assess the survivability of *L. acidophilus* during storage and to evaluate the effect of prebiotics on the survival of *L. acidophilus* in human being. The protein content and melting quality of the ice cream samples showed a significant (P<0.01) difference. The added prebiotics in the ice cream mix significantly (P<0.01) improved the growth of *L. acidophilus*. A significant reduction in the count of *L. acidophilus* was observed after freezing the ice cream mix and during 7 and 15 day of storage. Faecal examination of human volunteers fed with synbiotic ice cream for an experimental period of 15 days carried out on 0,7,15 and 21 days post treatment showed significantly increased faecal *L. acidophilus* count and significantly (p<0.01) reduced pH and coliform count. It may be concluded that the ice cream can be used as an excellent medium to deliver probiotics and prebiotics, to increase the activity of the *L. acidophilus* thereby improving gut health of the consumers.

Keywords: Synbiotic ice cream, *Lactobacillus acidophilus*, prebiotic substances, human studies

Introduction

The development of new food product turns out to be increasingly challenging, as it has to fulfill the consumer's expectations for products that are simultaneously relishing and healthy. Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Prebiotics are 'non-digestible or low-digestible food ingredients that benefit the host organism by selectively stimulating the growth or activity of one or a limited number of probiotic bacteria in the colon' (Gibson and Roberfroid, 1995). Probiotics and prebiotics may be combined to form synbiotic products that will benefit more consumers with health benefits (Frost and Sullivan, 2003). Ice cream has only nutritional significance but possesses no therapeutic value. Ice cream serves 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). Increased consumer interest in improving overall health and reducing risk for specific diseases has fuelled the demand for functional foods and beverages that provide health benefits beyond their traditional nutritional value. And accordingly the present study is envisaged to prepare whey protein enriched synbiotic ice cream by substituting whey protein concentrate (WPC) which harbours nutritionally and functionally superior whey proteins for skim milk and also incorporating prebiotic substances such as honey, fructo-oligosaccharides (FOS) and inulin along with probiotic bacteria *Lactobacillus acidophilus* in ice cream and to assess the survivability of *L. acidophilus* during storage and to evaluate the effect of prebiotics on the survival of *L. acidophilus in* human being.

Materials and Methods

Fresh cow milk was procured from the livestock farm, Veterinary College and Research Institute, Namakkal, Tamil Nadu state, India. Inulin obtained from Himedia Laboratories Private Limited, Mumbai, India, Fructo-oligofructose (FOS) obtained from the Kanisshka Flora Chem India, Chennai and honey obtained directly from honey hives, were

*Corresponding author.
Email: *ch.pandiyan@gmail.com*
Tel: 0091 94435 82717

utilized as prebiotic substances in the study. WPC (82 per cent protein) were procured from Kanishka Flora Chem (India), butter and SMP were procured from Aavin Dairy, Salem, Tamil Nadu state, India, stabilizer , emulsifier, sugar, and vanilla flavour were procured from the local market. Freeze dried culture of *Lactobacillus acidophilus* NCDC 14, which has proven therapeutic benefits, was obtained from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, Haryana, India. The treatments were divided in to TC (control ice cream), T1 (Probiotic ice cream- Ice cream + *L. acidophilus),* T2 (Ice cream + WPC + *L. acidophilus*), T3 (Ice cream + WPC + Honey+ *L. acidophilus*), T4 (Ice cream + WPC + Oligofructose+ *L. acidophilus*) and T5 (Ice cream + WPC + Inulin+ *L. acidophilus*).

Preparation of ice cream samples

The quantities of ingredients for the respective treatments are presented in Table 1. For each treatment, 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 WPC, SMP, sugar, stabilizer and emulsifier with constant stirring to dissolve the constituents completely. The ice cream mix was pasteurized at 80°C for 30 minutes and mixes were cooled to 5°C and aged overnight at the same temperature (Arbuckle, 1972). 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 probiotic culture *L. acidophilus* sub cultured in skimmed milk 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.

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 as described by Bodyfelt *et al. (1*988). Maximum scores allotted for flavour, body and texture, melting quality, colour, appearance and packaging (CAP) and bacterial count were 10, 5, 3, 5 and 2 respectively. Full marks (2/2) were given for bacterial count in the score card. Statistical analysis of data of the four replications was carried out by using completely randomized design (Table 2).

Table 1. Quantity of ingredients for 1000 g of ice cream mix for various treatments

Ingredients (g)	TC	T1	T2	T3	T4	T5
Milk	711.67	711.67	711.67	711.67	711.67	711.67
SMP	46	46	41.4	41.4	41.4	41.4
WPC	0	0	4.6	4.6	4.6	4.6
Butter	89	89	89	89	89	89
Sugar	150	150	150	150	150	150
Stabiliser & Emulsifier	3.33	3.33	3.33	3.33	3.33	3.33
*Prebiotics (%)	0	-	-	3	3	3
**L acidophilus* (%)	-	4	4	4	4	4
Total	1000	1000	1000	1000	1000	1000

*Addition of prebiotics and *L. acidophilus* are not taken for figuring of ice cream mix.

Table 2. Properties of experimental ice creams

Parameters	Control	T1	T2	T3	T4	T5
Total solids % (n=4)	36.13 ±0.08	36.13 ±0.03	36.10 ±0.04	36.18 ±0.03	36.15 ±0.06	36.13 ±0.05
Fat % (n=4)	10.05 ±0.06	10.05 ±0.03	10.03 ±0.02	10.10 ±0.04	10.05 ±0.06	10.13 ±0.05
Protein % (n=4)	4.73^{a} ±0.01	4.72^{a} ±0.01	5.07^{a} ±0.02	5.10^{a} ±0.02	5.13^{a} ±0.03	5.09^{a} ±0.01
Melting quality (in minutes) (n=4)	7.10^{a} ±0.06	6.42^{b} ±0.05	6.38^{b} ±0.04	6.38^{b} ±0.04	6.38^{b} ±0.03	6.38^{b} ±0.04
Coliform count (cfu/ml) (n=4)	1.52^{a} ±0.01	1.46^{b} ±0.01	1.47^{b} ±0.01	1.46^{b} ±0.01	1.48^{b} ±0.01	1.47^{b} ±0.01
Sensory analysis (n=6)						
Flavour (10)	8.80^{a} ±0.08	8.25^{b} ±0.11	8.22^{b} ±0.10	8.28^{b} ±0.10	8.22^{b} ±0.06	8.25^{b} ±0.11
Body and Texture (5)	4.10 ±0.10	4.10 ±0.10	4.10 ±0.24	4.00 ±0.16	4.10 ±0.10	3.88 ±0.17
Colour, Appearance and Packaging (5)	4.18 ±0.05	4.07 ±0.03	4.03 ±0.04	4.00 ±0.13	4.08 ±0.06	3.97 ±0.11
Melting Quality (3)	2.93 ±0.04	2.83 ±0.11	2.92 ±0.05	2.87 ±0.06	2.82 ±0.05	2.85 ±0.05
Bacterial Count (2)	2	2	2	2	2	2
Total score (25)*	22.08^{a} ±0.19	21.32^{b} ±0.22	21.25^{b} ±0.25	21.15^{b} ±0.30	21.25^{b} ±0.10	20.97^{b} ±0.27

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

The total solids and fat content of control and experimental probiotic and synbiotic ice cream were analyzed as per the Indian Standard (1964) and maintained at 10 and 36 per cent respectively, so as to produce ice cream with proper body and texture. The ice cream samples were analyzed for protein content as per the Indian Standard (1964). The melting quality of ice cream 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 *Lactobacillus acidophilus* in the ice cream mix and ice cream was evaluated (Day 0, 7 and 15) by using Reinforced clostridial agar with bromocresol green and clindamycin (RCBC) by serial dilution technique (Darukaradhya *et al.,* 2005).

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 (Lidbeck *et*

al., 1987). The volunteers subjected to investigate the effect of consumption of probiotic and synbiotic ice cream were free from gastrointestinal complaints, were not taking any medication associated with gastro intestinal activity, had not taken any antibiotic during the preceding two weeks prior to starting the study and the food did not contain any prebiotic or probiotic 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 faecal pH, *L. acidophilus* and coliforms were analysed on day 0 pretreatment and on days 7 and 15 post-treatment and from day 16 feeding ice cream samples were discontinued. Finally on day 21, again the pH, *L. acidophilus* and coliforms were analysed on the stool samples of the human volunteers. 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 10^9 using normal saline. One milliliter of faecal slurry from the dilutions 10^5 and 10^6 each were plated for enumeration of *L. acidophilus* using RCBC agar. Bacterial colony counts were made according to the colonial morphology and total log count per gram of sample (Darukaradhya *et al.,* 2005). Coliform count of the faecal sample was analysed as per the procedure described by Pandiyan and Geevarghese (2003).

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 Indian Standard (1964) and shown in Table 2. The fat and total solids content of the ice cream samples showed no significant difference between control and treatments. Addition of milk fat and solids to ice cream mix provides a smoother product and richness in mouth feel and also reduces the ice crystal size Arbuckle (1972). The increase in the protein content in the respective treatments is due to the addition of WPC, are shown in shown in Table 2. Hofi *et al.* (1993) reported that inclusion of WPC in ice cream mix improved the melting quality. Addition of Whey protein concentrate to frozen desserts produces favorable sensory and textural qualities (Tirumalesha and Jayaprakasha, 1998). In this study, addition of WPC improved the textural characteristics of the ice cream samples.

Melting quality of the probiotic and synbiotic ice cream samples (Table 2) had a ($P<0.01$) significant difference as compared to control. A faster melting rate was noticed 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 (Salem *et al*., 2005). Our results are also in agreement with the findings of Akalin and Erisir (2008) who found that the first dripping time was longer in probiotic ice creams supplemented with oligofructose and inulin in comparison with the control. Salem *et al. (*2006) noticed that the ice cream samples with probiotic culture (*L. acidophilus*) showed a faster melting rate than control. Trindade *et al.* (2007) produced the ice cream with different starter cultures (*Lactobacillus acidophilus* 74 - 2*, L. acidophilus* LAC 4 and yoghurt starter culture), fermenting up to a final pH 4.5 and observed a lower melting rates for probiotic fermented yellow mombin ice cream. 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 (Table 2). The coliform count in the different treatments was within the limit as prescribed by the Indian Standard (1964).

The mean flavour score showed a significant ($P<0.01$) difference between the control and treatments with probiotic bacteria. The body and texture, CAP and melting quality scores for the control and treatments showed no significant difference. There was a significant difference ($P<0.01$) in the total score of control and treatments shown in Table 2. All the probiotic and synbiotic ice cream samples scored slightly lower values in flavour scores and total scores than the control. Even though the probiotic and synbiotic ice cream samples exhibited probiotic flavour, which was acceptable and gave a good total impression with no marked off flavour by the sensory panel (Akin *et al*., 2007; Hagen and Narvhus, 1999).

The *L. acidophilus* count ($\log_{10}$cfu/ml) of control and treatment ice cream mixes (Table 3) showed a ($P<0.01$) significant difference among them. The treatments containing prebiotic substances had a higher count of *L. acidophilus* as compared to control. Addition of prebiotic substances has influence on the *L. acidophilus* growth in the ice cream mix. Survival of *L. acidophilus* La-5 and *B. animalis* Bb-12 were significantly enhanced with oilgofructose and the recommended minimum limit of 10^6 cfu/g was maintained for *B. animalis* Bb-12 in only probiotic ice cream with oligofructose during 90 days of storage (Akalin and Erisir, 2008). Hagen and Narvhus (1999), who found that the initial freezing in the ice cream freezer followed by hardening at -18°C to -23°C caused a reduction of less than one log cycle in the *L. acidophilus* count was observed (Hekmat and McMahon, 1992). The numbers of probiotic bacteria decreased by 0.7 to 0.8 log unit in ice cream

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

Groups	Count log_{10}cfu/ml and 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	9.45^{a} ±0.08	8.80^{a} ±0.28	8.50^{a}±0.15	8.23^{a} ±0.10
T4	9.50^{a} ±0.09	8.89^{a} ±0.13	8.55^{a} ±0.26	8.41^{a} ±0.23
T5	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)

during freezing or shortly afterwards (Hagen and Narvhus, 1999). During 12 weeks of frozen storage of probiotic ice cream using *L. acidophilus,* the count decreased by 2.23 log cfu/g, due to freezing, the bacterial cells might have injured and caused the death of the cells. However, the mechanical stresses of the mixing, freezing process and incorporation of oxygen in to the mix would have also resulted further decrease in the bacterial count (Salem *et al.,* 2005). In all the ice cream samples with probiotic bacteria stored at -18°C to -23°C for a period of 15 days, the viable count of *L. acidophilus* remained above the recommended minimum limit of 10^6 cfu/g. 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 (Haroldo *et al*., 2007).

The control and treatment ice cream samples fed to the human volunteers, the faecal pH values on day 0 had no significant difference (Table 4). The pH values had a significant difference (P<0.01) between control and treatments on day 7 and 15. The pH of human faeces after one week of ingestion of synbiotic fermented milk (FOS and *Lactobacillus casei)* was significantly (P<0.01) lower and daily consumption of fermented milk (80 ml) containing Lactobacillus (10^{10}) and FOS (2.5g) had significantly reduced stool pH, one week after consumption (Shamala *et al*., 2000; Shioiri *et al*., 2006). The synbiotic milk containing *L. acidophilus* (10^7 cfu/ml) and *B. lactis* (10^7cfu/ml) and 2 per cent inulin reduced in the pH of the stool samples in human volunteers (Casiragi *et al*., 2007). 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 and the existing pH values (Shamala *et al*., 2000; Shioiri *et al*., 2006; Casiragi *et al*., 2007). The mean pH of the faecal samples collected from the human volunteers on day 21 for the control and treatments showed no significant difference between the treatments.

The *L. acidophilus* count (log_{10} cfu/g) of the faecal samples collected from different treatment groups are presented in Table 5. *L. acidophilus* count of the faecal samples collected from different treatment

Table 4. pH of faecal samples collected from human volunteers fed with experimental ice cream

Day	Tc	T1	T2	T3	T4	T5
0	6.64 ±0.02	6.77 ±0.06	6.78 ±0.05	6.76 ±0.04	6.79 ±0.05	6.80 ±0.02
7	6.61^{a} ±0.04	5.80^{b}±0.02	5.66^{c} ±0.02	5.68^{c} ±0.01	5.68^{c} ±0.01	5.65^{c} ±0.01
15	6.65^{a}±0.03	5.59^{b} ±0.02	5.45^{c} ±0.02	5.47^{c} ±0.01	5.44^{c} ±0.01	5.43^{c} ±0.01
21	6.68 ±0.02	6.74 ±0.02	6.73 ±0.03	6.76 ±0.01	6.76 ±0.02	6.74 ±0.02

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

Table 5. *Lactobacillus acidophilus* count of faecal samples collected from human volunteers fed with experimental ice cream

Day	TC	T1	T2	T3	T4	T5
0	5.61±0.03	5.65±0.01	5.65±0.01	5.62±0.01	5.64±0.02	5.62±0.02
7	5.63^{c}±0.03	6.64^{ab}±0.04	6.68^{a}±0.03	6.69^{a}±0.04	6.75^{a}±0.02	6.76^{a}±0.02
15	5.63^{c}±0.04	6.64^{b}±0.04	6.64^{b}±0.03	7.14^{a}±0.20	7.20^{a}±0.14	7.16^{a}±0.15
21	5.67^{b}±0.06	6.76^{a}±0.05	6.80^{a}±0.05	6.74^{a}±0.05	6.78^{a}±0.05	6.80^{a}±0.06

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

groups of the human volunteers on 0 day did not differ significantly. A significant (P<0.01) difference was found between control and the treatment groups on day 7 and 15 and 7 day after cessation of feeding was noticed. The present results correlate with Langlands *et al*. *(*2000) that *in vivo* inulin feeding significantly increased the lactobacillus count in gut epithelium. These findings also correlate with the findings of Shioiri *et al*. (2006), that the faecal count of *Lactobacillus* after one week of ingestion of synbiotic fermented milk beverage was significantly higher than control. Synbiotic milk containing *L. acidophilus* (10^7 cfu/ml) and *B. lactis* (10^7 cfu/ml) and 2 per cent inulin fed to human volunteers showed an enhanced growth of bifidobacteria and lactobacilli (Casiragi *et al*., 2007). Greenbaum and Aryana (2006) reported that honey to increase (0.7 per cent w/v mix) counts of probiotics namely Bifidobacteria and Lactobacilli in the colon. Oral administration of *L. acidophilus* resulted in elevated stool levels throughout the period of dosing in healthy human volunteers, but the levels decreased after cessation of dosing on day 15. The present study indicates that consumption of probiotics either *L. acidophilus* along with WPC and prebiotic substances increased the faecal lactobacillus count as compared to control group. Hence, the prebiotics can be incorporated to improve the growth and survival of probiotic organism in the gastrointestinal tract in human being.

The mean coliform count (log_{10}cfu/g) of faecal samples collected from human volunteers is presented in Table 6. Statistical analysis of the data on day 0 had no significant difference in the coliform count between the groups. The mean coliform count (log_{10}cfu/g) of faecal samples collected on 7^{th} and 15^{th} day of feeding had a significant difference (P<0.01) between control and treatments. Feeding of probiotic and synbiotic ice cream samples to the human volunteers had significant reduction in the coliform count in the faecal samples obtained from the

human volunteers. Further, after cessation of feeding experimental ice cream samples on day 15, and the faecal samples analyzed after 7 days the coliforms had a significant reduction in the count. The results of our study are in agreement with Kleessan *et al.* (1997) who reported that inulin not only increased the probiotic count but also decreased the Enterococci and Enterobacteria counts in faeces. Further, Tuohy *et al*. (2006) who found that consumption of fermented milk containing *L. casei shirota* reduced the number of *E.coli* on day 7. Reduction in the coliform count in the faeces might be due to competitive inhibition of implantation and exclusion of coliform bacteria by the Lactobacilli as stated by Apella *et al.* (1992), who found that *L. acidophilus* had adhesion and competitive exclusion properties against pathogenic *E. coli*, *L. monocytogenes*, *Shigella sonnei*, and *S. typhimurium*. Low coliform count in the faeces of synbiotic ice cream fed individuals may also be due to possible lactic acid and bacteriocin production by the lactobacilli as per the findings of Nowroozi *et al*. (2004). Oh *et al.* (2000) found that *L. acidophilus* produced bacteriocin like substance which exhibits broad spectrum of antibacterial activity. The present results are corroborative to the finding of Shoaf *et al.* (2006) who observed that prebiotic oligosaccharides have anti-adhesive activity and thereby directly inhibit the adherence of pathogens to the host epithelial cell surface.

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

Day	Tc	T1	T2	T3	T4	T5
0	6.78±0.02	6.76±0.02	6.77±0.03	6.77±0.03	6.78±0.02	6.74±0.02
7	6.84^{a}±0.02	5.95^{b}±0.18	5.92^{b}±0.20	5.90^{b} ±0.18	5.82^{b} ±0.19	5.78^{b} ±0.21
15	6.62^{a}±0.02	5.73^{b}±0.17	5.70^{b}±0.20	5.68^{b}±0.18	5.60^{b} ±0.19	5.56^{b} ±0.21
21	6.60±0.02	6.58±0.02	6.59±0.03	6.59±0.02	6.63±0.02	6.56±0.01

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

Conclusion

Ice cream samples were prepared with WPC and prebiotic substances with *L. acidophilus*. An acceptable pH of 5.5 was achieved and the number *of L. acidophilus* in the synbiotic and probiotic ice cream was above the therapeutic levels (10^6) during processing and storage up to 15 days at -18ºC to 23ºC. The synbiotic and probiotic ice cream samples consumed by human volunteers could significantly improve the gut health by reducing the pH and number of coliforms of the faecal samples. There was a significant in the faecal lactobacilli count was noticed in the synbiotic and probiotic ice cream fed groups. Since, the ice cream is delicious product consumed by all age groups; it can be used as a medium for the growth and transfer of probiotic bacteria as well as prebiotic substances to maintain the normal flora and also for restoration of the gut microbes in combating the gut associated illness.

References

Arbuckle, W. S. 1972. Ice cream, AVI Publ Co Inc. Westport, Connecticut, 55-65.

Akalin, A.S. and Erisir, D.2008. Effects of Inulin and Oligofructose on the Rheological Characteristics and Probiotic Culture Survival in Low-Fat Probiotic Ice Cream. Journal of Food Science 73: 184-188.

Akin, M.B., Akın, M.S. and Kırmaci, Z. 2007. Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice- cream. Food Chemistry 104: 93–99.

Apella, M. C., Gonzalez, S. N., Nader de Macias, M. E., Romero, N. and Oliver, G.1992. *In vitro* studies on the inhibition of the growth of *Shigella sonnei* by *Lactobacillus casei* and *Lactobacillus acidophilus*. Journal of Applied Bacteriology 73: 480-483.

Antunes, E.C.A., Cazetto, T.F. and Bolini, H.M.A. 2005. Viability of micro-organism during storage, post acidification and sensory analysis of fat-free yogurts with whey protein concentrate. International Journal of Dairy Technology 58: 169-173.

Bodyfelt, F. W., Tobias, J. and Trout, G.M.1988. The sensory evaluation of dairy products. AVI Publ. New York.

Casiragi, M.C., Canzi, E., Zanchi, R., Donati, E. and Villa, L .2007. Effects of symbiotic milk product on human intestinal system. Journal of Applied Microbiology 103: 499- 506.

Darukaradhya, J., Phillips, M. and Kailasapathy, K. 2005. Selective enumeration of *Lactobacillus acidophilus*, *Bifidobacterium* spp., starter lactic acid bacteria and non-starter lactic acid bacteria from Cheddar cheese International Dairy Journal 16: 439 – 445.

FAO/WHO (Food and Agricultural Organisation/World Health Organisation). 2002. Guidelines for the evaluation of probiotics in food. Working group report. *www.fao.org*.

Frost, A. and Sullivan, S. 2003. European and United States Probiotic Market. Available at *http://www.marketresearch.com/product/display.asp?product.*

Gaon, D., Gracia, H., Winter, L., Rodriguez, N., Quintas, R., Gonzalez, S.N. and Oliver, G. 2003. Effect of *Lactobacillus acidophilis* and *Saccharomyces boulardii* on persistent diarrhea in children. Medicina (Buenos Aires) 63: 293-29.

Gibson, G .R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125: 1401–1412.

Greenbaum, A. and Aryana, K.J. 2006. Probiotic ice cream manufactured with honey, a natural sweetener with several health benefits. Journal of Dairy Science 90: 239- 242.

Hagen, M. and Narvhus, J.A. 1999. Production of ice cream

containing probiotic bacteria. Milchwissenschaft 54: 265- 268.

Hekmat, S. and McMahon, D.J. 1992. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. Journal of Dairy Science 75: 1415–1422.

Haroldo, M., Costa, S.S.M., Flores,M. and Pizarro, O. 2007. Viability of microorganism (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12) in ice cream. Iranian Journal Public Health 33: 1 -7.

Hofi, M., Fayed, A., El-Awamry, Z. and Hofi, A.1993. Substitution of non- milk fat solids in ice cream with ultra filtered whey protein concentrate. Egyptian Journal of Dairy Science 21: 139- 145.

Huse, P.A., Towler, C. and Harper, W.J. 1984. Substitution of non-fat milk solids in ice cream with ultra filtered whey protein concentrate and hydrolyzed lactose. NewZealand Journal of Dairy Science and Technology 19: 255-261.

Indian standard. (1964). 2802 - 1964. Indian standard for ice cream, Indian Standard Institution, New Delhi, India.

Khillari, S.A. and Zanjad, P.N., Rathod, K.S. and Raziuddin, M. 2007. Quality of low fat ice cream made with incorporation of whey protein concentrate. Journal of Food Science and Technology 44: 391-393.

Kleessen, B., Sykura, B., Zunft, J.H. and Blaut, M.1997. Effects of inulin and lactose on faecal microflora, microbial activity and bowel habit in elderly constipated persons. American Journal of Clinical Nutrition 65: 1397–1402.

Langlands, S.J., Hopins, M.J. and Cummings, J.H. 2000. Inulin and FOS feeding modify colonic mucosal bacteria *in vivo*. Gastroenterology 118: A772.

Lidbeck, A., Gustafsson, J.A. and Nord, C. E.1987. Impact of *Lactobacillus acidophilus* supplements on the human oropharyngea and intestinal microflora. Scandinavian Journal of Infectious Disease 19: 531–537.

Muse, M.R. and Hartel.,R. W. 2004. Ice Cream Structural Elements that Affect Melting Rate and Hardness. Journal of Dairy Science 87:1–10.

Nowroozi, J., M. Mirzaii and M. Norouzi. 2004. Study of *Lactobacillus* as Probiotic Bacteria. Iranian Journal of Public Health 33: 1-7.

Oh, S., Kim, S.H. and Worobo, R. W. 2000. Characterization and purification of a bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus*. Journal of Dairy Science 83: 2747–2752.

Pandiyan, C. and Geevarghese, P.I. 2003. Microbiological and sensory qualities of flavoured filled yoghurt incorporating coconut cream at different levels. Indian Journal of Dairy and Biosciences 15: 98-100.

Salem, M. M. E., Fathi, F.A. and Awad, R. A. 2005. Production of probiotic ice cream. Polish Journal of Food Nutrition Science 14/55: 267-271.

Salem, M.M.E., Fathi, F.A. and Awad, R. A. 2006. Production of functional ice cream. Deutsche Lebensmittel-Rundschau 102: 326-330.

Shioiri, T., Yahagi, K., Nakayama, S., Asahaa, T., Yuki, N., Kawakami, K., Yamaoka, Y., Sakai, Y., Nomoto, K. and Totani, M. 2006. The effects of a synbiotic fermented milk beverage containing *lactobacillus casei* strain shiriota and transgalatosylated oligosaccharides on defecation frequency, intestinal microflora, organic acid concentration, and putrefactive metabolites of sub-optimal health state volunteers. Bioscience Microflora 25: 137-146.

Shoaf, K., George, L. M., Armstrong, D. G. and Robert, W. H. 2006. Effect of prebiotic oligosaccharides on *E. coli.* Infection and Immunology 1128: 1030-1036.

Shamala, T. R. Y., Jyothi, S. and Saibaba, P. 2000. Stimulatory effect of honey on multiplication of lactic acid bacteria under *in vitro* and *in vivo* conditions. Letters in Applied microbiology 30: 453-455.

Tirumalesha, A. and Jayaprakasha, H. M. 1998. Effect of admixture of spray dried whey protein concentrate and butter milk powder on physico-chemical and sensory characteristics of ice cream. Indian Journal of Dairy Science 51:13–19.

Trindade, F.C.S., De, J.C.P., Balieiro, C., Dias, F.F., Sanino, A. and Boschini, C. 2007. Effects of culture, pH and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow mombin (*Spondias mombin* L) ice creams. Food Science and Technology International 13: 285- 291.

Tuohy, M.K., Pinart-Gilberga, M., Jones, M., Hoyles, L., McCartney, A.L. and Gibson, G.R. 2006. Survivality of a probiotic *Lactobacillus casei* in the gastro intestinal tract of healthy human volunteers and its impact on the faecal microflora. Applied Environmental Microbiology 102: 1026-1032.