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Study the Possibility of Manufacturing Therapeutic Ice Cream by Adding Synbiotic and Study its Microbiological and Sensory Characteristics

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Abstract

The study was conducted to prepare control, probiotic (*Lactobacillus acidophilus*), and synbiotic (*L. acidophilus* and inulin) ice cream, *L. acidophilus* content, pH, titratable acidity, sensory properties were evaluated during frozen storage periods. *L. acidophilus* counts were the higher in synbiotic ice cream, adding inulin to probiotic ice cream enhanced significantly (P<0.05) the content of *L. acidophilus*. Freezing process caused a decrease in *L. acidophilus* counts along with storage periods in all the samples of ice cream. Synbiotic ice cream was the lower in pH values and the higher in TA values compared to the other ice cream samples. Synbiotic ice cream was the better in overall acceptance followed by probiotic and control ice cream, respectively. So, ice cream fortification with *L. acidophilus* probiotic bacteria and prebiotic inulin have a positive influence on all sensory characteristics. Probiotic content of both synbiotic and probiotic ice cream could be considered as functional therapeutic healthy product since it was more than the lowest concentration of probiotic bacteria to provide the beneficial attributes which are 10^6 cfu/g at the consumption time of the product.

Keywords: *L. acidophilus*, Inulin, synbiotic ice cream, probiotic ice cream, viable counts

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INTRODUCTION

Ice cream is a nutritious frozen milk-based product, eaten widely all over the world for its taste and the nutritional value. The pH of ice cream is about[6-7] which regards as a neutral pH, with long storage period. Dairy products represent an ideal source and good media to carry probiotic bacteria into the intestinal tract of human because it provides the required favorable environment which improves probiotic bacteria growth (Salem et al., 2005; Darukaradhya et al., 2006; Homayouni et al., 2008). Probiotics are live microorganisms which when administrated in adequate amount confer healthy effects on the human (Hill et al., 2014). Probiotics enhance the balance of the intestinal microbial content, stimulate the growth of the good bacteria over the intestinal bad bacteria and then improving the host health and lowering the diseases of GIT when an adequate amount is consumed (≥ 10^6 cfu/g) (Bansal et al., 2016; Sangsila et al., 2016). While prebiotics definition is: food ingredients that are indigestible and have a positive effect on the health of the host through stimulating the activity and the growth of the beneficial bacteria of the intestinal tract (Zhang et al., 2018). Many researchers have studied probiotic bacteria survivability in ice cream manufactured in different techniques, such as cultured and nonfermented ice cream mixtures (Akın 2005; Turgut and Cakmakci 2009; Pandiyan et al. 2012). Adding of probiotics and prebiotics to ice cream will add a nutritious value by improving its functional properties (Balthazar et al., 2015; Ozturk et al., 2018). Thus, prebiotics are added to ice cream to boost the growth and the activity of probiotic bacteria in ice cream components. At consumption time, the most important value of dairy products is the viable number of probiotics bacteria which determines the product efficiency (Mortazavian and Sohrabvandi 2006). So, to get the consumers' trust in ice cream and dairy probiotic products, it is very important to keep an acceptable level of bacteria during the production and the shelf life period of the product (Saxelin et al. 1999). The survivability and metabolic activity of probiotic bacteria should be maintained in every step of ice cream manufacturing till the ingestion time by consumers, in addition to keeping their ability to survive in GIT (Sanz, 2007). Many researchers have evaluated probiotic bacteria lowest concentration to provide the positive attributes which is 10^6 cfu/g at the consumption time of the product (Kurmann and Rasic 1991; Rybka and Kailasapathy 1995; Blanchette et al. 1996; Gomes and Malcata 1999). Also, the ingestion of 10^8-10^9 cfu per day of probiotic bacteria is required to provide desirable healthy effects to humans (Vinderola et al. 2000; Oliveira et al. 2001). Producing synbiotic or probiotic ice cream with good organoleptic characteristics is not easy and needs a technical knowledge by processors (Aryana & Summers, 2006; Favaro-Trindade et al., 2006). Nevertheless, probiotic bacteria will not modify the product's sensory characteristics intensely (Champagne et al., 2005). Although, it could be producing a desired quality of probiotic and synbiotic ice cream (Vardar & Öksüz, 2007).

Study aims to manufacture control, probiotic (*L. acidophilus*), synbiotic (probiotic *Lactobacillus acidophilus* and prebiotic inulin) ice cream, and study probiotic bacteria content, pH, titratable acidity and the sensory properties (flavor and taste, body and texture, and color and appearance) over a 28 day of storage period, and subsequently the possibility of manufacturing therapeutic ice cream and extending its shelf life.

MATERIALS AND METHODS

Ice cream manufacturing

Three ice cream samples (3 kg for each sample) were manufactured. The mixtures components as shown in Table 1, were homogenized together according to Arbuckle (1986) with some modification, after that mixtures were heated up to 80°C for 30 second, then cooled to 5°C, left at 5°C overnight for aging. Control ice cream prepared without any further addition.

Table 1. Ice cream mixtures components

Ingredients	Control ice-cream	Probiotic ice cream	Synbiotic Ice cream
Milk fat %	8	8	8
Milk solid non-fat (MSNF) %	12	12	12
Sugar	16	16	16
Stabilizer	0.3	0.3	0.3
Emulsifier	0.2	0.2	0.2
Vanilla flavor %	0.3	0.3	0.3

Probiotic ice cream sample was made by adding (1% v/v) *L. acidophilus* log 9 cfu/ml. Synbiotic ice cream sample was made by adding (1% v/v) *L. acidophilus* log 9 cfu/ml in addition to 2% inulin. Each sample then divided into (100 ml) plastic covered cup and kept in freezer at -18 °C during the storage periods of 28 days.

Probiotic bacteria enumeration

Ten grams of ice cream were diluted in (100 ml) sterilized peptone water (0.1%), 1 ml of the diluted ice cream was poured on (MRS) NNLP agar, where NNLP is (nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate) (Tharmaraj and Shah, 2003). Incubation was conducted under anaerobic conditions at 37°C for 72 h. The results of enumeration were calculated as (cfu/g) colony forming units/gram of sample. All samples enumerations were conducted in triplicate.

Titratable acidity and pH determination

A hundred gram of ice cream from each sample was melted at 5°C, then acidity was measured by titration the melted ice cream samples against 0.1N NaoH and using phenolphthalein as indicator. The pH was measured using pH meter.

Sensory evaluation

Sensory evaluation was accomplished by 5 specialists and based on the following rating scales: Flavor and taste (1-10), body and texture (1-5), and color and appearance (1-5) and conducted weekly during the storage periods (Homayouni et al., 2005).

RESULTS AND DISCUSSION

Table 2 illustrates the changes in *Lactobacillus acidophilus* counts of probiotic and synbiotic ice cream samples throughout 28 days of storage periods. The viable counts of *L. acidophilus* were 7.95 and 7.98 log cfu/g in probiotic and synbiotic ice cream samples, respectively at zero time, and decreased gradually during the storage periods to reach 6.95 and 7.21 log cfu/g for probiotic and synbiotic ice cream samples, respectively at storage periods' end. *L. acidophilus* counts in both probiotic and synbiotic samples were higher than Prashanth et al., (2018) who found that *L. acidophilus* counts ranged between 6.12 and 6.91 log cfu/g in ice cream synbiotic sample. The mean of synbiotic *L. acidophilus counts* sample was higher than the mean of probiotic ice cream sample. Inulin addition to ice

Table 2. Viable counts of *L. acidophilus* in probiotic and synbiotic ice-cream samples during storage periods

L. acidophilus counts	0	7	14	21	28	Mean - sample
Probiotic ice-cream	7.9500 ±0.05000	7.4900 ±0.06000	7.2700 ±0.02000	7.1100 ±0.03000	6.9500 ±0.03606	7.3540 ±0.36134
Synbiotic ice-cream	7.9800 ±0.01000	7.5700 ±0.05292	7.4100 ±0.01000	7.3200 ±0.04000	7.2100 ±0.01732	7.4980 ±0.27896
Mean-day	7.9650 ±0.03619	7.5300 ±0.06693	7.3400 ±0.07797	7.2150 ±0.11929	7.0800 ±0.14464	
rlsd	treat 0.091		day 0.1250		Treat*day 0.261	

Table 3. pH and TA of control, probiotic and synbiotic ice-cream samples during storage periods

Sample	pH values					
	0	7	14	21	28	Mean - sample
Control ice-cream	6.5600 ±0.0200	6.5000 ±0.0100	6.5100 ±0.0360	6.4900 ±0.0000	6.4900 ±0.0100	6.5100 ±0.03162
Probiotic ice-cream	6.5000 ±0.0000	5.9300 ±0.0173	5.8000 ±0.0100	5.7400±0.02646	5.7000 ±0.0100	5.9340 ±0.30409
Synbiotic ice-cream	6.5300 ±0.0100	5.8500 ±0.0000	5.7300 ±0.0173	5.6600 ±0.0100	5.5900 ±0.0100	5.8720 ±0.35208
Mean-day	6.5300 ±0.0282	6.0933 ±0.3071	6.0133±0.3743	5.9633 ±0.3967	5.9267 ±0.4252	6.1053 ±0.39189
rlsd	treat 0.0620*		day 0.0367		Treat*day 0.126	
Sample	TA					
	0	7	14	21	28	Mean - sample
Control ice-cream	0.2600±0.00000	0.2800±0.01000	0.2700±0.00000	0.2900±0.01000	0.2800±0.00000	0.2760±0.01183
Probiotic ice-cream	0.2800±0.01000	0.3800±0.00000	0.4000±0.01000	0.4200±0.00000	0.4300±0.01000	0.3820±0.05609
Synbiotic ice-cream	0.2600±0.01000	0.4000±0.00000	0.4200±0.01000	0.4400±0.01000	0.4700±0.00000	0.3980±0.07561
Mean-day	0.2667±0.01225	0.3533±0.05590	0.3633±0.07089	0.3833±0.07089	0.3933±0.08689	
rlsd	treat 0.0160*		day 0.0100		Treat*day 0.0210	

cream (synbiotic) sample significantly (P<0.05) improved probiotic *L. acidophilus* growth, and there were significant (P<0.05) differences in *L. acidophilus* counts between probiotic and synbiotic ice cream samples on day 14 until the end of the storage periods. The increase in *L. acidophilus* counts of synbiotic compared to probiotic samples in this study may be attributed to prebiotic inulin which acts as a protective mechanism during the storage (Prashanth et al*.,* 2018), and this in one line with Pandiyan et al. (2012a) who found that adding FOS to ice cream mixture significantly (P<0.01) enhanced *L. acidophilus* cultures' growth. Furthermore, inulin enrichment to ice cream affected significantly on probiotic bacteria counts (Akin et al*.,* 2007). Furthermore, ice cream enriched with inulin improved significantly (P<0.01) *L. acidophilus* growth (Pandiyan et al., 2012b). Also, study results agreed with Ayar et al. (2018) who reported that adding inulin and fibers of grain and fruit to ice cream samples affected positively on *L. acidophilus* counts, inulin ice cream was the higher in *L. acidophilus* counts followed by fibers of grain and fruit ice cream compared to the negative control. Inulin could be used by probiotic bacteria as a source of energy (Rastall, 2010), and this may clarify the reason behind increasing *L. acidophilus* counts in inulin (synbiotic) ice cream sample. On the contrary, there wasn't any effect for inulin on the survivability of *L. casei* 01 of synbiotic ice cream during the passage time via artificial GIT environment (Balthazar et al., 2018). There was a significant decrease in *L. acidophilus* counts in probiotic ice cream sample along with storage periods comparing with a limited significant decrease for *L. acidophilus* counts in synbiotic ice cream sample until the day 14 which was non-significant reduction till the end of storage periods. The reduction in the microorganisms' numbers, resulting from freezing, is probably because of freeze injury which led to cells death. Although the decrease in the counts of probiotic *L. acidophilus*, the manufactured probiotic and synbiotic ice cream samples might be regarded as a probiotic diet along with the 28 days of the storage period, since *L. acidophilus* counts remained above the minimum limits of 10^6 cfu/g at product consumption' time (Vinderola & Reinheimer, 2000; Argentina, 2013). Likewise, Balthazar et al., (2018) found that all control, probiotic and synbiotic ice cream mixtures improved the growth of *L. casei* 01 and kept its counts higher than the minimum limits (log 6 cfu/ml) to be therapeutically during storage. In same line, Akalin and Erisir (2008) reported that many studies have been conducted in different places in the world, results showed that probiotic cultures had the ability to keep their stability in frozen manufactured food, and lost minimum of their viability.

Table 3 shows the pH and titratable acidity (TA) values of control, probiotic and synbiotic manufactured ice cream during storage periods. At zero time, pH values were 6.56, 6.50, and 6.53, while TA values were 0.26, 0.28, and 0.26 for control, probiotic and synbiotic ice-cream samples, respectively. pH values decreased while TA values increased gradually during storage periods to reach 6.49, 5.70, and 5.59 for pH and 0.28, 0.43, and 0.47 for TA values for control, probiotic and synbiotic ice-cream samples, respectively after 28 days of storage. For pH values, the lowest pH value was synbiotic ice cream sample at ending of storage period followed by probiotic and control ice cream samples, respectively. While for TA values, synbiotic ice cream sample also was the highest TA at ending of storage period followed by probiotic and control ice cream samples, respectively.

Ice cream enriched with probiotic bacteria is more acid than the control ice cream, because of lactic acid production via probiotic bacteria (Basyigit et al., 2006). While, addition of inulin to ice cream fortified with probiotic bacteria encouraged the activity of probiotic bacteria and subsequently decreased the pH and increased the TA. This finding was in agreement with Guler-Akin et al. (2016) who reported that there was a reduction in the pH and an increase in the titratable acidity in carob extract and whey powder enriched-ice cream samples, the metabolic activity of probiotic bacteria was stimulated by adding the extract of carob and the powder of whey. Also, Ayar et al. (2018) attributed the probiotic ice cream protective affects to the fiber addition which led to the relatively low pH. Clear significant differences (P < 0.05) were found among all samples for pH and TA values, which means that addition of probiotic bacteria and prebiotic affected significantly on ice cream pH and TA. Control ice cream values ranged between 0.26 and 0.28 and these results were close to Barman et al. (2017) who found that the titratable acidity of ice cream samples in Kolkata and its Suburbs ranged from 0.235% to 0.275% lactic acid. The relatively low pH and high TA did not affect somehow on probiotic *L. acidophilus* since the resistance to pH and acidity is strain-dependent in probiotic bacteria, and it has been found that *L. acidophilus* has a wide cytoplasmic buffering capacity (3.72–7.74) of pH, which enables it to be stable and resistible to the cytoplasmic pH changing under the acidic environment (Godward et al. 2000; Tamime et al. 2005). The pH values mean of synbiotic and probiotic ice cream samples ranges from 5.87 to 5.93 during frozen storage and this in one line with Thaochalee et al. (2018) who reported that the mean pH values of the synbiotic ice creams range from 5.75 to 5.95 during frozen storage, and this is within the optimal growth pH range for *L. acidophilus* (5.5-6.0) as reported by Mohammadi et al. (2011). Also, the titratable acidity mean increased from 0.2760 in control ice cream to 0.3980 in synbiotic ice cream and this was in line with Prashanth et al. (2018) who reported that elevation of TA means correlated with increasing the levels of prebiotic green banana flour, this elevation may be attributed to the various organic acids compounds in prebiotic banana flour (Prashanth et al., 2018). The final pH of all ice cream samples was within the optimal limited for the product because of the ice cream is not counted as an acidic product, and the low

Table 4. Sensory properties of control, probiotic and synbiotic ice-cream samples during storage periods

Flavor and taste	Control Ice cream	Probiotic Ice cream	Synbiotic Ice cream	Mean-day
0	9.60 ±0.548	9.60 ±0.548	9.80 ±0.447	9.67 ±0.488
7	9.00 ±0.707	9.20 ±0.447	9.60 ±0.548	9.27 ±0.594
14	8.80 ±0.837	9.00 ±0.000	9.20 ±0.447	9.00 ±0.535
21	8.20 ±0.447	8.60 ±0.548	9.00 ±0.707	8.60 ±0.632
28	7.60 ±0.548	8.00 ±0.707	8.80 ±0.447	8.13 ±0.743
Mean - sample	8.64 ±0.907	8.88 ±0.726	9.28 ±0.614	
rlsd	treat	day		Treat*day
	0.40	0.47*		ns
Body and texture	**Control Ice cream**	**Probiotic Ice cream**	**Synbiotic Ice cream**	**Mean-day**
0	4.40 ±0.894	4.40 ±0.894	4.60 ±0.548	4.47 ±0.743
7	4.00 ±0.707	4.20 ±0.447	4.40 ±0.548	4.20 ±0.561
14	3.80 ±0.447	4.00 ±0.000	4.20 ±0.447	4.00 ±0.378
21	3.40 ±0.548	3.60 ±0.548	4.00 ±0.000	3.67 ±0.488
28	3.00 ±0.707	3.40 ±0.548	3.80 ±0.447	3.40 ±0.632
Mean - sample	3.72 ±0.792	3.92 ±0.640	4.20 ±0.500	
rlsd	treat	day		Treat*day
	0.46	0.47		ns
Color and appearance	**Control Ice cream**	**Probiotic Ice cream**	**Synbiotic Ice cream**	**Mean-day**
0	5.00 ±0.000	4.60 ±0.548	4.80 ±0.447	4.80 ±0.414
7	4.40 ±0.548	4.40 ±0.548	4.60 ±0.548	4.47 ±0.516
14	3.80 ±0.447	4.00 ±0.707	4.40 ±0.548	4.07 ±0.594
21	3.40 ±0.548	3.80 ±0.447	4.20 ±0.447	3.80 ±0.561
28	3.40 ±0.548	3.60 ±0.548	3.80 ±0.447	3.60 ±0.507
Mean - sample	4.00 ±0.764	4.08 ±0.640	4.36 ±0.569	
rlsd	treat	day		Treat*day
	0.35	0.40		ns

pH (4.0-4.5) values have undesirable effect on the acceptance characteristics, while study results showed that the final pH values ranged from 5.59 to 6.49 after 28 days of frozen storage.

Sensory properties evaluation (flavor and taste, body and texture, color and appearance) was conducted to all ice cream samples to determine the acceptance of ice cream samples as illustrated in Table 4. All tested sensory properties were close to each other at zero time for control, probiotic and synbiotic ice cream samples and ranged between 9.60-9.80 for flavor and taste, 4.40- 4.60 for body and texture, and 4.80 -5.00 for color and appearance. Along with increasing the storage periods, the sensory properties decreased slightly, where flavor and taste scored 7.60, 8.00 and 8.80, body and texture scored 3.00, 3.40 and 3.80, and color and appearance scored 3.40, 3.60 and 3.80 for control, probiotic and synbiotic ice cream samples, respectively after the frozen storage of 28 days.

The synbiotic ice cream was the higher scores in all sensory properties followed by probiotic and control ice cream samples, respectively, and this finding agreed with Thaochalee et al*.,* (2018) who found that the evaluated sensory characteristics showed a good acceptability for all synbiotic ice cream samples. Adding prebiotic inulin, affected positively on the sensory characteristics of synbiotic ice cream sample for the current study and this result agreed with Vardar and Oksuz (2007) who found that good sensory properties resulted from artisan strawberry ice-cream enriched with *L. acidophilus*. Also, adding unripe banana flour as prebiotic to probiotic ice cream gained the higher overall acceptability scores by the consumers (Prashanth et al*.*, 2018). Furthermore, Cruz et al., (2010) also assured that prebiotic ingredients supplemented to ice cream had a higher influence on texture and flavor. While, Vital et al*.,* (2018) illustrated that there were no significant differences resulted from the addition of the residue of grape juice on sensory properties of ice cream. Addition of *L. acidophilus* to ice cream affected positively on sensory properties of current study and this result resembles the finding of Vijayageetha et al. (2011) who reported that probiotic *L. acidophilus* and *Bifidobacterium* mixture ice cream was noted to has the higher flavor score. On contrary, a low effect on the texture, flavor or the other sensory properties have been found when ice cream fortified with probiotic bacteria (Mohammadi et al., 2011). Also, the sensory properties of ice cream have not affected by adding free and microencapsulated probiotics strains (EL-Sayed et al*.*, 2014).

The mean values of all tested sensory properties were the highest for synbiotic ice cream sample followed by probiotic and control ice cream samples, respectively. No significant differences ($P < 0.05$) were found among most of ice cream samples sensory properties especially during the first two weeks, while clear significant differences ($P < 0.05$) were found between synbiotic ice cream sensory properties and the control ice cream, and slightly between probiotic ice cream and control ice cream, while no significant differences ($P < 0.05$) were found between probiotic and synbiotic ice cream samples. These findings somehow, disagree with Ayar et al*.* (2018) who found that all sensory properties of ice cream samples were significantly ($P < 0.05$) influenced by adding different fiber material. Also on contrary with Pandiyan, (2014) who found a significant difference ($P<0.05$) among the treatments in the total sensory scores, while all probiotic and synbiotic ice cream samples recorded lower values in the scores of the total sensory comparing with the control. Furthermore, the sensory properties of probiotic (*Bifidobacterium* BB-12) ice cream enriched with xylitol, isomalt, maltitol and erythritol were inconsistent and not unanimous (Kalicka et al., 2019). The first indicator regarding food choice is the flavor, followed by the attributes health benefits, if the added supplements provide undesirable flavors to the product, consumers will never consume the therapeutic ice cream although this functional food will improve their health. Probiotic ice cream could provide different flavor properties comparing with the conventional one, while adding of inulin to ice cream will offer some suitable properties and these properties are responsible somehow for the sensory acceptance high score values of synbiotic ice cream.

CONCLUSION

The population of *L. acidophilus* in probiotic and synbiotic ice cream was higher than the therapeutic minimum level during the manufacturing and storage periods, so it could be count as probiotic food. Also, addition of inulin affected significantly on *L. acidophilus* counts and sensory properties in both probiotic and synbiotic ice cream. It is suggested that ice cream could be effectively used as a carrier to deliver probiotic bacteria in addition to prebiotic supplements such as inulin to improve the gut health of human. The success of therapeutic probiotic and synbiotic dairy products depends on consumer acceptance to such products in addition to its health benefits. So, paying more attention for the sensory properties in addition to the health benefits of these products as therapeutic products and functional food is the key to develop it. Thus, extra studies are required to evaluate the survival and diversity of probiotic bacteria in addition to looking for the best prebiotic to be enriched to gain the required viable number and preferred sensory properties for the product and subsequently for consumers.

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Not applicable.

CONFLICT OF INTEREST

We, the authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors Conceived and designed the analysis and collected the date while Al-Shawi SG. performed the analysis and wrote the paper. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

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