

Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream

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Abstract

Ice-cream containing probiotic bacteria was produced by mixing fortified milk fermented with probiotic strains with ice-cream mixes with different sugar concentrations (15, 18, 21% (w/w)). Cultures were grown (37 °C, 12 h) in UHT skimmed milk supplemented with or without inulin addition (1% and 2%). The fermented milk was added to the ice-cream mix to a level of 10% w/w.

Increasing sugar concentration stimulated physical and sensory properties. The addition of inulin improve viscosity, first dripping and complete melting times; however, it had no effect on the sensory properties. Viable bacteria numbers were highest at 18% sugar concentration. *Streptococcus thermophilus* was most stable in all the samples of probiotic ice-cream with $>10^7$ cfu g⁻¹ throughout the storage period. The count of *Lactobacillus delbrueckii* ssp. *bulgaricus* was reduced by 1.5 log cycles. The counts of *Lactobacillus acidophilus* and *Bifidobacterium lactis* decreased to 10^5 cfu g⁻¹ in the control samples, whereas the counts were 10^6 cfu g⁻¹ in the samples supplemented with inulin. The results suggested that the addition of inulin stimulated the growth of *L. acidophilus* and *B. lactis*, which resulted in an improved viability of these organisms.

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1. Introduction

In recent years, there has been a growing interest in using probiotic micro-organisms as dietary adjuncts in the dairy industry. Products have been developed and are on the market worldwide. Among dairy products with live cultures, probiotic ice-creams or fermented frozen desserts are also gaining popularity (Kailasapathy & Sultana, 2003). However, probiotic organisms are unstable in such products. The loss of viability of probiotic organisms in a frozen yogurt may be due to acidity, freeze injury and oxygen toxicity (Hekmat & McMahon, 1992; Ravula & Shah, 1998). Air incorporation is essential to obtain the desired overrun in ice-cream; however, excess oxygen will affect

growth of micro-aerophilic *Lactobacillus acidophilus* and anaerobic *Bifidobacteria* (Kailasapathy & Sultana, 2003). Laroia and Martin (1991), Hekmat and McMahon (1992), and Haynes and Playne (2002) reported satisfactory survival of probiotic bacteria in frozen dairy desserts.

The efficacy of added probiotic bacteria depends on dose level. Their viability must be maintained throughout the product's shelf-life and they must survive the gut environment. To exert positive health effects, they have to establish themselves in certain numbers in the gastrointestinal tract (Kailasapathy & Sultana, 2003). A standard, requiring a minimum of 10^6 – 10^7 colony forming units per gram (cfu g⁻¹) of *L. acidophilus* and/or *Bifidobacteria* in fermented milk products, has been introduced by several food organizations world-wide (IDF, 1992).

Consequently, manufacturers are interested in developing a process that can provide high densities of the

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probiotic strains in the product (Talwalkar & Kailasa-phy, 2004). For example, supplementing milk with a combination of protein hydrolysates, fructose whey protein concentrate, tomato juice and papaya pulp stimulated *L. acidophilus*, while cysteine, acid hydrolysates, tryptone, vitamins, dextrin and maltose improved the viability of *Bifidobacteria*. Probiotics, such as oligosaccharides, are added to food, mainly to allow the preferential growth of probiotic organisms (Lourens-Hattingh & Viljoen, 2001).

Inulin, a non-digestible carbohydrate containing naturally-occurring fructooligosaccharides, possesses some characteristics of dietary fibres, and as such is of particular interest for its metabolic properties (El-Nagar, Clowes, Tudorica, & Kuri, 2002). Besides its health benefits, inulin is also considered to have prebiotic properties such as the ability to stimulate probiotic bacteria without adversely affecting flavour (El-Nagar et al., 2002; Özer, Akın, & Özer, 2005).

The objective of this study was to produce a probiotic ice-cream containing high levels of probiotic bacteria ($>10^6$ – 10^7 cfu g⁻¹, which is the recommended minimum daily intake), and to determine their survival immediately after freezing and during storage. In an attempt to improve the survival of the cells during freezing, different levels of inulin were added to the batches. Furthermore, the effects of inulin and sugar levels on the physical and sensory characteristics of the probiotic ice-cream were also examined.

2. Materials and methods

2.1. Bacteria and growth conditions

Commercial freeze-dried mixed probiotic culture (code MYBIO2 Rhodia Food, France), consisting of *Streptococcus salivarius* spp. *thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus* LA-14 and *Bifidobacterium lactis* BL-01, was obtained from Rhodia Food, Turkey. The cultures were propagated in sterile, 12% reconstituted skim milk. The cultures were grown for 18 h at 37 °C, using a 0.03% (w/v) inoculum. For propagation of probiotic bacteria, sterile reconstituted skim milk was supplemented with 1 and 2% inulin (Sigma). The cultures were kept frozen until required for addition to the mixes prior to ice-cream manufacture. Before addition to ice-cream mix, each frozen culture combination had 130 ml of sterile UHT milk added and was gently swirled for 10 min to facilitate dispersal.

2.2. Procedure for ice-cream manufacturing

Cow's milk was supplied from the Animal Husbandry unit of the Faculty Agriculture, Harran University. The dry matter content in the milk was adjusted by adding non-fat milk powder (Pınar Dairy, Turkey). BIVI ICE Stick-DCT Guar gum (E 412), xanthan gum (E 415), carrageenan (E 407), sodium alginate (E 401), and dextrose (KATPA, Turkey) were used as stabilizers. Commercial

sugar was used as a sweetener, and vanilla was added for aroma development. Ice-cream was formulated with the following composition (percentage by weight) such that it had 30–36% total solids for a total batch of 100 kg:

Whole milk	45
Cream	15
Skim milk powder	7.4
Sugar	15, 18 or 21
Stabiliser	0.5
Vanillin	0.1
Water	10

The milk and cream (35% fat) were mixed and temperature brought to 45 °C; the skim milk powder and sugar, plus water, were added and pasteurized at 85 °C for 1 min. After addition of vanillin, the ice-cream mix was divided into three parts of 20 l each (A, B, C), followed by addition of 3% and 6% sugar to batches B and C, respectively. This mix was homogenised at 85 °C while still hot, and allowed to cool to 40 °C. Then each batch was divided into three parts of 6 l each (1, 2, 3) and the fermented milk, supplemented without or with 1 and 2% inulin, was added as a 10% addition to an ice-cream mix, followed by freezing. The probiotic ice-cream was produced by using a vertical freezing machine of 6 kg capacity (Uğur, Nazilli, Turkey). The partially frozen mix was packaged in 50, 100 and 200 ml cups and stored at –18 °C. Manufacturing stages for the production of probiotic ice-cream are shown in Fig. 1. The experiment was conducted in triplicate.

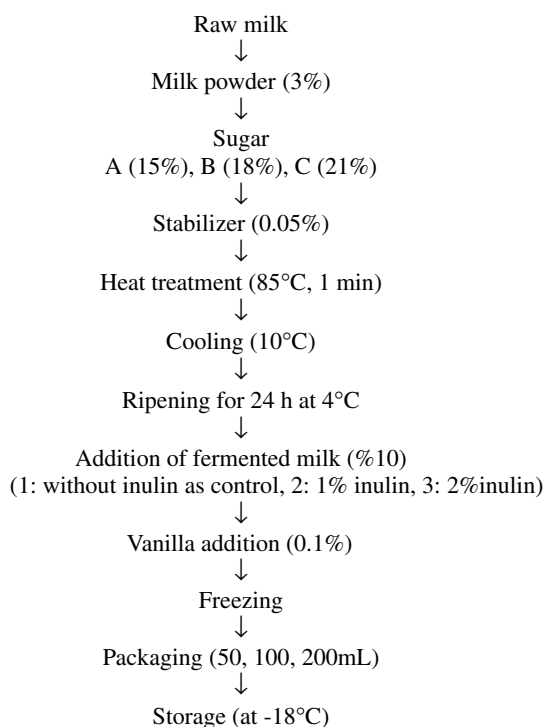


Fig. 1. Production of probiotic ice-cream.

2.3. Bacteriological analysis

Bacterial counts were determined after 12 h fermentation, immediately after mixing, and after freezing, at 7, 30, 60 and 90 days of storage at -18°C . Fermented milk, mix and ice-cream samples (10 g) were decimally diluted in 100 ml sterile peptone water (0.1%) and 1 ml aliquot dilutions were poured onto plates of the various selective and differential agars in triplicate. M17 agar was used for the enumeration of *S. thermophilus* (Rybka & Kailasaphaty, 1996) *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* was incubated by using MRS and MRS with sorbitol agar, respectively (Dave & Shah, 1996). Enumeration of *B. lactis* was achieved as described by Haynes and Playne (2002). All plates were incubated at 37°C for 72 h. M17 was incubated aerobically, whereas all other media plates were incubated anaerobically. Anaerobic conditions were created using Anaerocult A sochets (Merck). The results were expressed as colony-forming units per gram (cfu g^{-1}) of sample.

2.4. Chemical analysis

The pH of the milk and ice-cream was measured using a digital pH-meter and titratable acidity was measured by titrating 10 g of sample with 0.1 N NaOH using phenolphthalein as indicator (TSE, 1994). The dry matters of milk and ice-cream were determined by drying samples at $100 \pm 1^{\circ}\text{C}$ for 3.5 h using an air oven (TSE, 1994). The fat content of milk was determined by the Gerber method (TSE, 1994). Acetaldehyde was quantified by static headspace using Agilent 6890 GC coupled to a headspace sampler, according to Ott, Germond, Baumgartner, and Chaintreau (1999).

2.5. Physical measurements

The overrun of the final product was determined using the formula (Akin, 1990):

Overrun

$$= \frac{\text{Weight of unit mix-weight of equal volume of ice-cream}}{\text{Weight of equal volume of ice-cream}} \times 100$$

First dripping and complete melting times were measured according to Güven and Karaca (2002). 25 g of tempered samples were left to melt (at room temperature, 20°C) on a 0.2 cm wire mesh screen above a beaker. First dripping and complete melting times of samples were determined as seconds.

The viscosities of the ice-creams were determined at 4°C using a digital Brookfield Viscometer, Model DV-II (Brookfield Engineering Laboratories, Stoughton, MA, USA) (Özer, Robinson, Grandison, & Bell, 1997).

2.6. Sensory assessment

The samples were organoleptically assessed by ten panellists, using a sensory rating scale of 1–10 for flavour and

taste, and 1–5 for consistency and 1–5 for colour and appearance, as described by Bodyfelt, Tobias, and Trout (1988). The properties evaluated included: (a) six attributes for flavour and taste (no criticism: 10, cooked flavour: 9–7, lack of sweetness and too sweet: 9–7, lack of flavour: 9–6, yogurt/probiotic flavour: 8–6, acidic/sour: 8–6, rancid and oxidized: 6–1, and other: 5–1), (b) eight characteristics of body and texture (no criticism: 5, crumbly: 4–2, coarse: 4–1, weak: 4–1, gummy: 4–1, fluffy: 3–1, sandy: 2–1) and (c) four terms describing colour and appearance (no criticism: 5, dull colour: 4–1, non-uniform colour: 4–1, unnatural colour: 3–1). The panel of assessors was an external panel of non-smokers who were very familiar with dairy products and were checked on the basis of sensory acuity and consistency.

Physical, chemical and sensory analyses were carried out 1 week after production.

2.7. Statistical analysis

All statistical analyses were carried out using the SPSS statistical software programme (version 5.0). Statistically different groups were determined by the LSD (least significant difference) test (Düzgüneş, Kesici, Kavuncu, & Gürbüz, 1987).

3. Results and discussion

3.1. Chemical and physical characteristics

The gross chemical composition of the cow's milk used in the production of probiotic ice-creams was: pH 6.60 ± 0.017 , titratable acidity $6.55 \pm 0.029^{\circ}\text{SH}$, dry matter $12.09 \pm 0.036\%$ and fat $3.03 \pm 0.058\%$. The dry matter contents (%) of the ice-cream mixes were: A1 30.58 ± 0.036 , A2 30.52 ± 0.026 , A3 30.37 ± 0.066 , B1 33.61 ± 0.053 , B2 33.63 ± 0.026 , B3 33.58 ± 0.035 , C1 36.67 ± 0.000 , C2 36.66 ± 0.046 , C3 36.49 ± 0.030 .

The initial pH of milk (6.59–6.62 at 0 h) (data not shown) decreased to 5.81–6.00 during probiotic ice-cream making. It was observed that acidity increased as the inulin level increased ($p < 0.01$). We concluded that an increase in inulin content of the fermented milk had stimulated the metabolic activities of starter bacteria and improved development of acidity.

Acetaldehyde contents (ppm) of the samples were: A1 1.7 ± 0.06 , A2 1.7 ± 0.01 , A3 1.7 ± 0.02 , B1 1.7 ± 0.05 , B2 1.7 ± 0.05 , B3 1.8 ± 0.02 , C1 1.7 ± 0.07 , C2 1.7 ± 0.07 , C3 1.7 ± 0.03 . The effect of sugar level and addition of inulin on the acetaldehyde was insignificant ($p > 0.05$).

The physical properties of probiotic ice-creams are shown in Table 1. Overrun values rose from 34.0 to 37.5%, as sugar content increased from 15% to 21% ($p < 0.01$). Similar results were found in frozen yogurts by Güven and Karaca (2002). It was found that the addition of inulin had an insignificant effect on overrun values of the ice-cream samples ($p > 0.05$).

Table 1
Physical properties of probiotic ice-creams ($n = 3$)

Ice-cream ^A	Overrun values (%)	Firts dripping times (s)	Complete melting times (s)	Viscosity (cP)
A1	34.1 ± 0.300 ^c	1780 ± 18.0 ^f	5224 ± 20.0 ^{bc}	1074 ± 18.0 ^f
A2	34.3 ± 0.300 ^c	1800 ± 5.0 ^f	5195 ± 50.0 ^c	1107 ± 40.0 ^f
A3	34.7 ± 0.608 ^c	1921 ± 18.5 ^d	5247 ± 9.54 ^b	1191 ± 9.54 ^c
B1	35.8 ± 0.200 ^d	1895 ± 13.2 ^e	5186 ± 40.0 ^c	1205 ± 23.0 ^{de}
B2	36.0 ± 0.361 ^{cd}	1887 ± 11.4 ^e	5200 ± 30.0 ^{bc}	1238 ± 2.00 ^d
B3	36.5 ± 0.436 ^{bc}	2004 ± 15.7 ^b	5313 ± 3.61 ^a	1327 ± 5.29 ^c
C1	36.7 ± 0.520 ^{ab}	1961 ± 16.8 ^c	4806 ± 20.0 ^e	1398 ± 21.0 ^b
C2	36.3 ± 0.458 ^{bcd}	1950 ± 8.89 ^c	4840 ± 30.0 ^e	1404 ± 19.0 ^b
C3	37.3 ± 0.265 ^a	2058 ± 11.4 ^a	5107 ± 9.85 ^d	1512 ± 4.58 ^a

^{a-c} Means in the same column followed by different letters were significantly different ($p < 0.01$).

^A A1: 15% sugar without inulin, A2: 15% sugar supplemented with 1% inulin, A3: 15% sugar supplemented with 2% inulin, B1: 18% sugar, without inulin, B2: 18% sugar supplemented with 1% inulin, B3: 18% sugar supplemented with 2% inulin, C1: 21% sugar, without inulin, C2: 21% sugar supplemented with 1% inulin, C3: 21% sugar supplemented with 2% inulin.

The first dripping times were prolonged as sugar contents increased in the samples ($p < 0.05$). Our results indicated that addition of inulin at a rate of 1% had an insignificant effect on the first dripping time ($p > 0.05$). However, addition of 2% inulin to ice-creams led to an increase in first dripping time. The longest complete melting time was in the samples with 15% sugar. The complete melting time was found to be related to the sugar content ($p < 0.01$). Sugar concentration affected the complete melting time negatively. Our results indicate that increased additions of inulin to ice-cream mixes increased complete melting times. The results for melting characteristics suggest that inulin may act as a stabiliser due to its capacity for binding water. The result of this is that the water molecules become immobilized and unable to move freely among other molecules of the mix. The ice-cream melting process may also be described in relation to the freedom of movement of molecules; inulin (with its ability to reduce the free movement of water molecules) appears to retard product melting (El-Nagar et al., 2002). El-Nagar et al. (2002) also reported that addition of inulin reduced melting rate of yog-ice-cream.

The viscosity of the samples increased as the sugar content increased ($p < 0.01$). The addition of inulin caused an increase in the viscosity ($p < 0.05$). Viscosity values slightly increased with increasing inulin levels. This can be explained by the interactions of the dietary fibre and liquid components of ice-cream. Inulin, being highly hygroscopic, would bind water. Similar results were reported by El-Nagar et al. (2002).

3.2. Bacterial counts

Fig. 2 shows the variations in bacterial counts in fermented milk (12 h; milk fermented for 12 h), mix and ice-cream, with and without inulin addition. During freezing, the numbers of viable yogurt and probiotic bacteria decreased by 0.6–0.93 and 1.09–1.12 log units, respectively, and the numbers in the frozen ice-cream were found to be

in the range of 8.18, 8.22 and 8.22, 8.55, 8.59, 8.59, 7.99, 8.19 and 8.19 log cfu g⁻¹ for *S. thermophilus*, 7.60, 7.68, 7.67, 7.78, 7.79, 7.79, 7.52, 7.50 and 7.50 log cfu g⁻¹ for *L. delbrueckii* ssp. *bulgaricus*, 7.70, 7.91, 7.99, 7.89, 8.03, 8.08, 7.64, 7.88 and 7.88 log cfu g⁻¹ for *L. acidophilus*, 7.78, 7.96, 8.06, 7.93, 8.09, 8.14, 7.73, 7.94 and 7.94 log f cfu⁻¹ or *B. lactis* in the samples A1, A2, A3, B1, B2, B3, C1, C2 and C3, respectively.

The decline in bacterial counts, as a result of freezing, is likely due to the freeze injury of cells, leading eventually to the death of cells. However, the mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mix, may have resulted in a further decrease in bacterial count. Similar results were reported by Ravula and Shah (1998), Shah and Ravula (2001) and Haynes and Playne (2002).

The highest viable bacteria numbers were in the samples with 18% sugar. The addition of inulin had no effect on numbers of *S. thermophilus* or *L. delbrueckii* ssp. *bulgaricus*, while the survival of *L. acidophilus* and *B. lactis* was improved. The numbers of *L. acidophilus* and *B. lactis* increased as inulin level increased ($p < 0.01$), due to the possible prebiotic effects of inulin. Inulin, at a rate of 2% and 5.9 pH, gave the best prebiotic effect. Palframan, Gibson, and Rastall (2003) reported similar results.

In nine ice-cream samples, the numbers of *S. thermophilus* remained high until the end of the storage period. There was an approximately 1.5 log cycle decrease in the counts of *L. delbrueckii* ssp. *bulgaricus*. Davidson, Duncan, and Hackney (2000) found no significant decrease in *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* numbers in fermented frozen yogurt. In contrast, Miles and Leeder (1981) found that *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* concentrations decreased by an average of 1.5 and 0.5 log cfu ml⁻¹, respectively, when stored for 2 weeks at -28.9 °C in frozen yogurt. Lopez, Medina, and Jordano (1998) observed only a slight decline in lactic acid bacteria in three batches (pH = 4.32, 5.09 and 5.53) of commercial frozen yogurt stored at

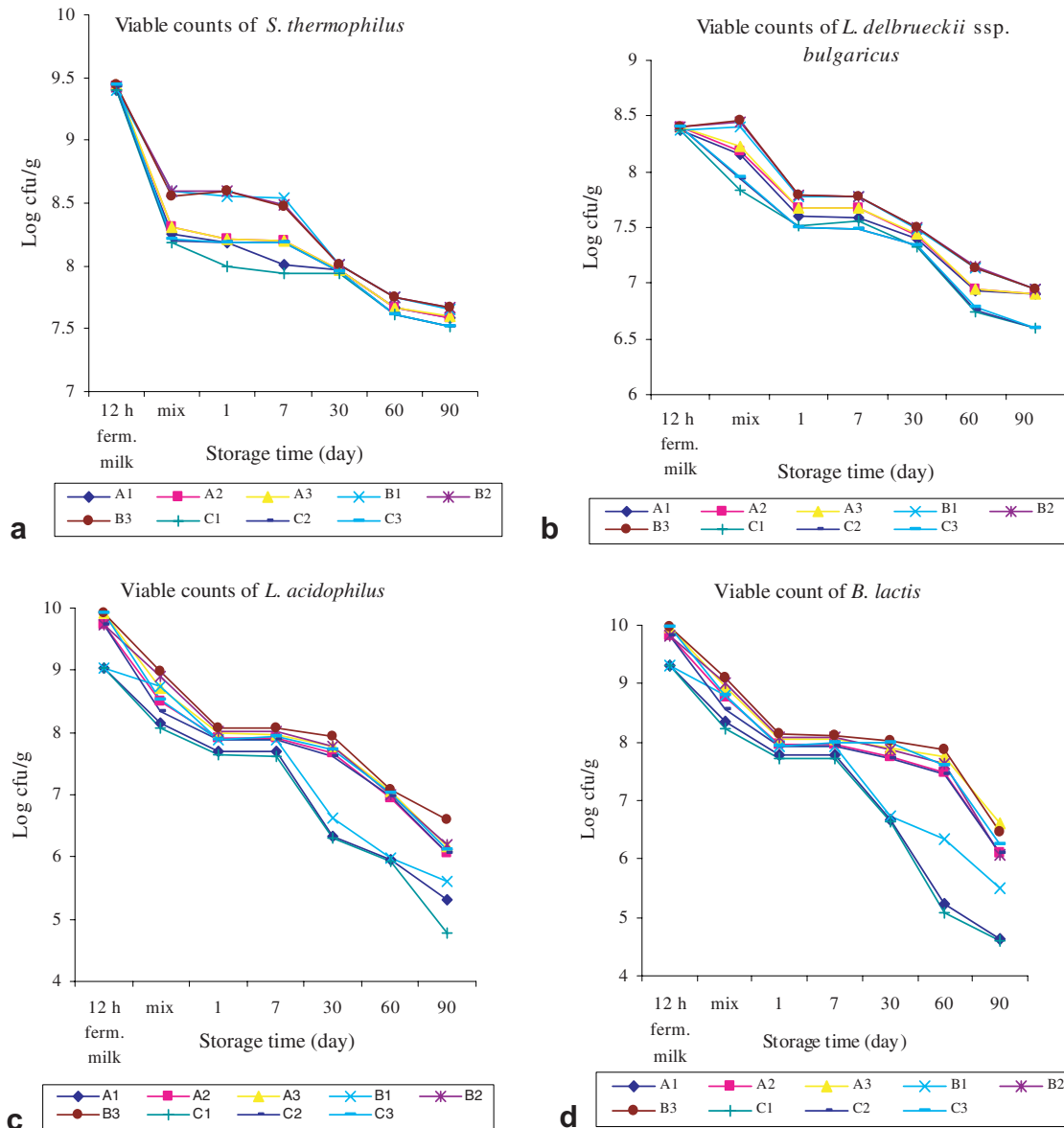


Fig. 2. Viable counts of *S. thermophilus* (a), *L. delbrueckii ssp. bulgaricus* (b), *L. acidophilus* (c) and *B. lactis* (d) in ice-cream with different sugar concentrations and with or without inulin addition during 90 days storage at -18°C . Note: A1: 15% sugar without inulin, A2: 15% sugar supplemented with 1% inulin, A3: 15% sugar supplemented with 2% inulin, B1: 18% sugar, without inulin, B2: 18% sugar supplemented with 1% inulin, B3: 18% sugar supplemented with 2% inulin, C1: 21% sugar, without inulin, C2: 21% sugar supplemented with 1% inulin, C3: 21% sugar supplemented with 2% inulin.

-23°C for 1 year. They suggested that, because of the close link of streptococci and lactobacilli in fermentation, both types of organisms were important in survival of bacterial cultures.

L. acidophilus and *B. lactis* decreased rapidly in control samples. The viable counts were 105 cfu g^{-1} by the end of the 90 day storage period. The decrease in the numbers of *L. acidophilus* and *B. lactis* in samples supplemented with inulin was less than those of the control samples and the viable count at the end of 90 days storage period was 106 cfu g^{-1} . In all samples, the number of *B. lactis* was higher than that of *L. acidophilus*. Hekmat and McMahon (1992) found that the viability of *L. acidophilus* in a stan-

dard ice-cream mix decreased by 2 log cycles after storage for 17 weeks at -29°C .

Since ice-cream is a whipped product, oxygen is incorporated in large amounts. *Bifidobacterium* is strictly anaerobic; therefore, oxygen toxicity may be a major factor of cell death (Kailasapathy & Sultana, 2003). Kailasapathy and Sultana (2003) reported that the viability of *B. lactis* decreased by 1.8 and 2.4 log cycles, respectively, when present in non-fermented types of ice-creams. Haynes and Playne (2002) found that in a low fat ice-cream, *B. lactis* BLC-1 survived better than did *L. acidophilus* Lafti™ L10 and *L. paracasei* subsp. *paracasei* LCS-1 over 52 weeks at -25°C .

Table 2
Organoleptic properties of probiotic ice-cream^A

Samples ^B	Colour and appearance	Body and texture	Flavour and taste	Total
	(1–5)	(1–5)	(1–10)	(1–20)
	Organoleptic properties/scores			
A1	4.21 ± 0.026 ^c	4.43 ± 0.020 ^b	8.43 ± 0.062 ^d	17.07 ± 0.069 ^d
A2	4.18 ± 0.050 ^c	4.38 ± 0.040 ^b	8.45 ± 0.050 ^d	17.01 ± 0.123 ^d
A3	4.20 ± 0.020 ^c	4.40 ± 0.056 ^b	8.50 ± 0.050 ^d	17.10 ± 0.104 ^d
B1	4.50 ± 0.030 ^a	4.53 ± 0.000 ^a	9.13 ± 0.030 ^a	18.16 ± 0.030 ^a
B2	4.45 ± 0.052 ^{ab}	4.55 ± 0.017 ^b	9.10 ± 0.108 ^{ab}	17.99 ± 0.087 ^{bc}
B3	4.40 ± 0.070 ^b	4.40 ± 0.070 ^a	9.15 ± 0.026 ^a	18.10 ± 0.144 ^{ab}
C1	4.35 ± 0.070 ^b	4.56 ± 0.036 ^a	8.95 ± 0.060 ^c	17.91 ± 0.098 ^c
C2	4.44 ± 0.053 ^{ab}	4.58 ± 0.098 ^a	9.03 ± 0.030 ^c	18.05 ± 0.111 ^{abc}
C3	4.45 ± 0.070 ^{ab}	4.55 ± 0.020 ^a	9.00 ± 0.000 ^c	18.00 ± 0.056 ^{abc}

^{a-c} Means in the same column followed by different letters were significantly different ($p < 0.01$).

^A Mean values from 10 panellists.

^B A1: 15% sugar without inulin, A2: 15% sugar supplemented with 1% inulin, A3: 15% sugar supplemented with 2% inulin, B1: 18% sugar, without inulin, B2: 18% sugar supplemented with 1% inulin, B3: 18% sugar supplemented with 2% inulin, C1: 21% sugar, without inulin, C2: 21% sugar supplemented with 1% inulin, C3: 21% sugar supplemented with 2% inulin.

3.3. Sensory evaluations

The sensory scores of the samples are given in Table 2. The points allocated for colour, body-texture and taste showed that an increase in sugar content brought about an improvement in the structure, creaminess, flavour and aroma of the products ($p < 0.01$). The addition of inulin had no effect on sensory properties of probiotic ice-creams ($p > 0.05$). A yogurt or probiotic flavour was not found to be particularly noticeable. One reason for this could be the high pH of the ice-cream. All the samples gave a good total impression, were medium sour and did not have any marked off-flavour during the storage period. None of the ice-creams were judged to be crumbly, weak, fluffy or sandy.

4. Conclusions

Effects of inulin and different sugar levels on physical and sensory characteristics of probiotic ice-cream were investigated. Increasing sugar concentration led to products with better physical and sensory properties. The addition of inulin improved viscosity, first dripping and complete melting times, but had no effect on sensory properties of the samples.

Survival of yogurt and probiotic bacteria was also studied in probiotic ice-cream with different sugar concentrations and with or without supplementary inulin during 90 days. Sugar concentrations significantly affected viable bacteria numbers ($p < 0.01$). The highest number was in the samples with 18% sugar. The survival of probiotic bacteria was higher in the samples supplemented with inulin. This could be due to prebiotic effects of this oligofructose. *S. thermophilus* was most stable in all the samples of probiotic ice-cream with $>10^7$ cfu g⁻¹ throughout the storage period. The counts of *L. delbrueckii* ssp. *bulgaricus* were reduced by 1.5 log cycles. The counts of *L. acidophilus* and *B. lactis* decreased to 10^5 cfu g⁻¹ in the control

samples, whereas the counts were 106 cfu g⁻¹ in the samples supplemented with inulin. The results suggested that the addition of inulin stimulated the growth of *L. acidophilus* and *B. Lactis*, which resulted in improved viability of these organisms.

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