

# Effects of ascorbic acid and glucose oxidase levels on the viability of probiotic bacteria and the physical and sensory characteristics in symbiotic ice-cream

doi: 10.15567/mljekarstvo.2015.0206

M. B. Akin\*, F. Dasnik

Harran University Agricultural Faculty, Department of Food Engineering,  
63040 Şanlıurfa, TurkeyPrispjelo - Received: 21.08.2014.  
Prihvaćeno - Accepted: 02.03.2015.

## Abstract

In this study, the effects of addition of different amounts of ascorbic acid and glucose oxidase on the properties of symbiotic ice cream were investigated. Ice-cream containing inulin (2 % (w/w)) was produced by mixing fortified milk fermented with probiotic strains with the ice-cream mixes containing different ascorbic acid and glucose oxidase concentrations (0.025, 0.05, 0.1 (w/w)). The cultures were grown (37 °C, 12 h) in UHT skimmed milk. The fermented milk was added to the ice-cream mix up to a level of 10 % w/w. Increasing the concentration of ascorbic acid stimulated the growth of *Lactobacillus acidophilus* LA-5 (*L. acidophilus*) and *Bifidobacterium animalis* subsp. *lactis* BB-12 (*Bifidobacterium* BB-12). On contrary, increasing the concentration of glucose oxidase negatively affected the growth of *L. acidophilus* and *Bifidobacterium* BB-12. However, both, ascorbic acid and glucose oxidase concentration had no effect on physical and sensory properties of ice cream. The results suggested that the addition of ascorbic acid stimulated the growth of *L. acidophilus* and *Bifidobacterium* BB-12 and could be recommended for ice cream production.

*Key words:* ice-cream, probiotic bacteria, ascorbic acid, glucose oxidase

## Introduction

Due to their attributed health benefits, probiotic bacteria (such as *L. acidophilus* and/or *Bifidobacteria*) have been increasingly applied in the dairy industry during the past two decades and are consumed at appropriate levels as part of a balanced diet. Many studies indicated that ice cream was an excellent vehicle for probiotic bacteria when compared to fermented dairy products. The pH of ice cream is higher than that of common fermented milks which is an important advantage over other dairy products (Da Silva et al., 2014). 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. Air incorporation is essential to obtain the desired overrun in ice cream, but excess oxygen will most probably affect the growth of microaerophilic strains such as *L. acidophilus* and anaerobic species like *Bifidobacteria* (Akin et al., 2007). In order to obtain therapeutic benefits, a suggested minimum level for probiotic bacteria in fermented milk was established and ranges from 10<sup>6</sup> to 10<sup>7</sup> CFU/mL (Güler-Akin and Akin, 2007; Rodrigues et al., 2011). Therefore, manufacturers were interested in developing a process that can provide high densities of the probiotic strains in the product.

Oxygen content and redox potential have been shown to be important factors affecting the viability

\*Corresponding author: Tel: +90 414 2470903; Fax: +90 414 2474480; E-mail: [mutluakin@harran.edu.tr](mailto:mutluakin@harran.edu.tr)

of bifidobacteria during storage of fermented milks. Ascorbic acid (vitamin C9) can act as an oxygen scavenger and might cause a smaller decrease in the probiotic bacteria counts during storage of yoghurt (Dave and Shah, 1997). The use of a glucose-glucose oxidase enzyme complex has been proposed as an effective alternative to reduce the oxygen dissolved in the product and consequently to maintain *Bifidobacterium longum* counts at levels capable of delivering benefits to the consumer. Using this approach and surface response methodology, predictive models were established and validated, and glucose oxidase was found to be a parameter of the greatest importance for predicting *B. longum* counts (Cruz et al., 2010, 2013). Consequently, the addition of glucose oxidase in higher concentrations was suggested since it might positively affect the probiotic characteristics of the product. The addition of glucose oxidase into yogurts during processing could be a potential option to decrease the oxidative stress in probiotic yogurts. In general, high counts of viable strains (*Lactobacillus acidophilus* and *Bifidobacterium longum*) were observed along the refrigerated storage (Cruz et al., 2012; Lollo et al., 2012; Cruz et al., 2013).

Storage period of the product should be characterised by oxygen level as low as possible to avoid toxicity and death of the microorganism and consequent loss of functionality of the product (Cruz et al., 2007, 2009). Oxygen permeation through the package may also have an adverse effect on probiotic viability (Ranadheera et al., 2013). The use of a plastic system with a low oxygen permeability transfer rate may decrease the amount of oxygen that passes the packaging system and gets in touch with the food matrix. Cruz et al. (2012) reported that there was a synergism between the packaging system and the glucose oxidase added to the products. By combining the use of packaging systems with different oxygen permeability rates with the addition of glucose oxidase, conditions which enable maintaining low levels of dissolved oxygen and appropriate the cell viability of *B. longum* and *L. acidophilus* up to the 21<sup>st</sup> day of storage, could be achieved.

This research aimed to evaluate the effect of increased levels of glucose oxidase and ascorbic acid on the viability of *L. acidophilus* and *Bifidobacterium* BB-12 in symbiotic ice cream. Furthermore, the effects of glucose oxidase and ascorbic acid on the

physical and sensory characteristics of symbiotic ice cream were also examined.

## Materials and methods

### Materials

The non-fat milk powder (96 % total solids) (Pinar Dairy, Turkey), cream (35 % fat) (Tat Can Industry. A.S. SEK Plant, Turkey) and *Lactobacillus acidophilus* LA-5 (*L. acidophilus*) and *Bifidobacterium animalis* subsp. *lactis* BB-12 (*Bifidobacterium* BB-12) (Peyma-Chr. Hansen, Turkey) were used for ice cream production. Carrageenan (E 407), Guar gum (E 412), Xanthan gum (E 415) and Sodium alginate (E 401), Karragenan (Sosa Ingredients, S.L. Ctra de Granera, Spain) were used as stabilizers. Lecithin was used as emulsifier and was obtained from Sosa Ingredients (S.L. Ctra de Granera, Spain). Sucrose (100 % TS) was used as a sweetener, and vanilla was added for aroma development. Ascorbic acid and glucose oxidase were obtained from Sigma Chemicals (İstanbul, Turkey). All of the other used reagents were of analytical grade.

### Preparation of fermented milk

The reconstituted skim milk with 12 % non-fat dry matter was made from skim milk powder and heated at 105 °C for 3 min. After the heating treatment, it was cooled at 37 °C and inoculated with probiotic cultures (*L. acidophilus* and *Bifidobacterium* BB-12) until pH of 4.7 was reached. Then it was cooled to 4±1 °C and used for ice cream production.

### Ice cream manufacture

Ice cream was formulated with the following composition (percentage by weight) such that it had 34-35 % total solids for a total batch for a total batch of 5 kg: 11 % non-fat total milk solid, 18 % sugar, 5 % fat, 0.8 % stabilizers, 0.3 % emulsifier and 2 % inulin.

The milk and the cream (35 % fat) were mixed warmed up to 45 °C. Subsequently the skim milk powder, sugar, stabilizers, emulsifier, inulin and plus water were added. The obtained mixture was pasteurized at 85 °C for 1 min, it was homogenised at

85 °C while still hot and afterwards cooled to 4 °C for ageing during the next 24 h. By the end of the aging process, 10 % (w/w) of fermented milk was added to the obtained ice-cream mix. Then the ice-cream mix was divided into seven parts of 5 kg each. The first batch was a control sample(A), the second (B), third (C) and fourth (D) batches were supplemented with glucose oxidase at a rate of 0.025 %, 0.05 % and 0.1 % respectively, while the fifth (E), the sixth (F) and the seventh (G) batches were supplemented with ascorbic acid at a rate of 0.025 %, 0.05 % and 0.1 %, and 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 into 100 mL cups and stored at -18 °C. The experiment was conducted in a duplicate.

#### *Chemical analysis*

The pH of the produced ice cream samples was measured using a digital pH-meter and titratable acidity was determined according to the Soxhlet-Henkel method (Guler-Akin and Akin, 2007). The dry matter content of ice-cream samples was determined by drying the samples at 105±1 °C for overnight to a constant weight using an air oven (T.S.E. 1989). The fat contents of ice cream samples were determined by the Gerber method (Guler-Akin and Akin, 2007).

The pH of the produced ice cream samples was measured using a digital pH-meter and titratable acidity was determined according to the Soxhlet-Henkel method (Guler-Akin and Akin, 2007). The dry matter content of ice-cream samples was determined by drying the samples at 105±1 °C for overnight to a constant weight using an air oven (T.S.E. 1989). The fat contents of ice cream samples were determined by the Gerber method (Guler-Akin and Akin, 2007).

#### *Physical measurements*

The overrun of the final product was determined formulas follows (Akin et al., 2007):

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

First dripping and complete melting times were measured according to Güven and Karaca (2002). Meltdown rate was conducted according to Da Silva et al. (2014). 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. The melted weight was recorded at the 30<sup>th</sup> and the 60<sup>th</sup> minute. 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) (Akin et al., 2007).

#### *Sensory assessment*

Sensory analysis of the ice-cream samples was assessed by ten untrained panellists. Thereat a 10 point hedonic scale was used to evaluate coldness, firmness, viscosity, smoothness, colour and appearance, mouth coating, flavour, taste and general acceptability (1 = strongly unacceptable, 10 = very good) as described by Aime et al. (2001). The panel of assessors was an external panel (consisted of staff from the Harran University Department of Food Engineering, Turkey) of non-smokers who were very familiar with dairy products and were checked on the basis of sensory acuity and consistency. Each panellist received 7 samples of ice cream to taste and to evaluate the sensory characteristics at each serving. Panellists were also instructed to consume water between the samples in order to maintain discretion. Physical, chemical and sensory analyses were carried out 1 week after the production.

#### *Bacteriological analysis*

Bacterial counts were determined after 12 h fermentation, immediately after mixing, and after freezing, and 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. *L. acidophilus* and *Bifidobacterium* BB-12 were incubated by using MRS with sorbitol and MRS-NNLP agar, respectively (Dave and Shah, 1996). All plates were incubated anaerobically at 37 °C for 72 h. Anaerobic conditions were created using Anaerocult A sachets (Merck). The results were expressed as colony-forming units per gram (cfu g<sup>-1</sup>) of sample.

### Statistical analysis

Statistical analysis of data via one-way analysis of variance (ANOVA) was performed to check the significance of differences at  $p < 0.05$  using SPSS Version 5.0 (SPSS Inc. Chicago, IL, USA).

### Result and discussions

#### Bacterial counts

Figure 1 and 2 present the variations in bacterial counts of fermented milks (12 h; milk fermented for 12 hours), mixes and symbiotic ice-creams.

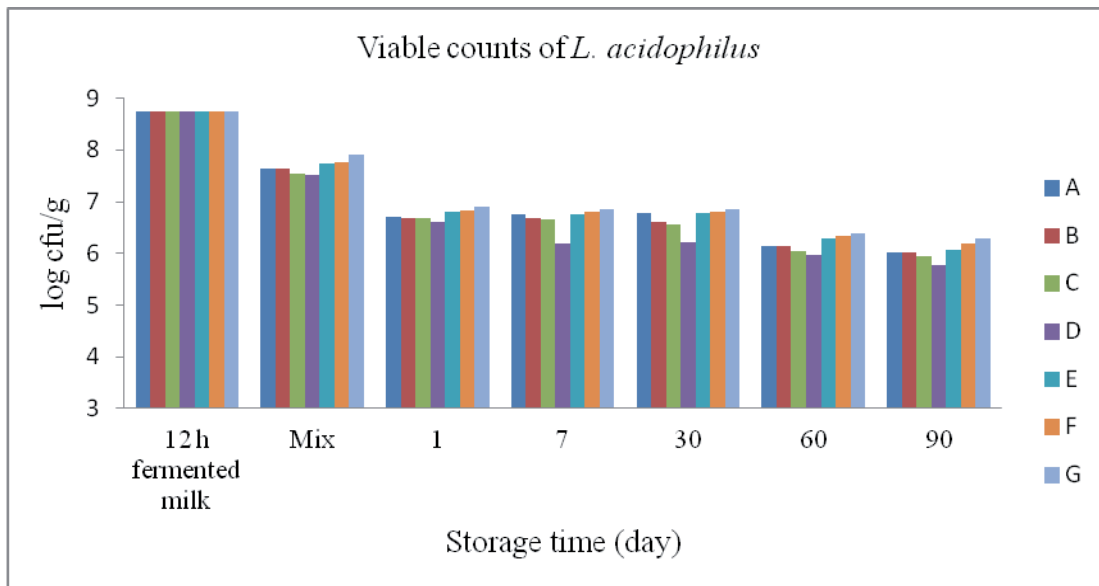


Figure 1. Viable counts of *L. acidophilus* in symbiotic ice-cream with different glucose oxidase and ascorbic acid concentrations during 90 days storage at  $-18\text{ }^{\circ}\text{C}$

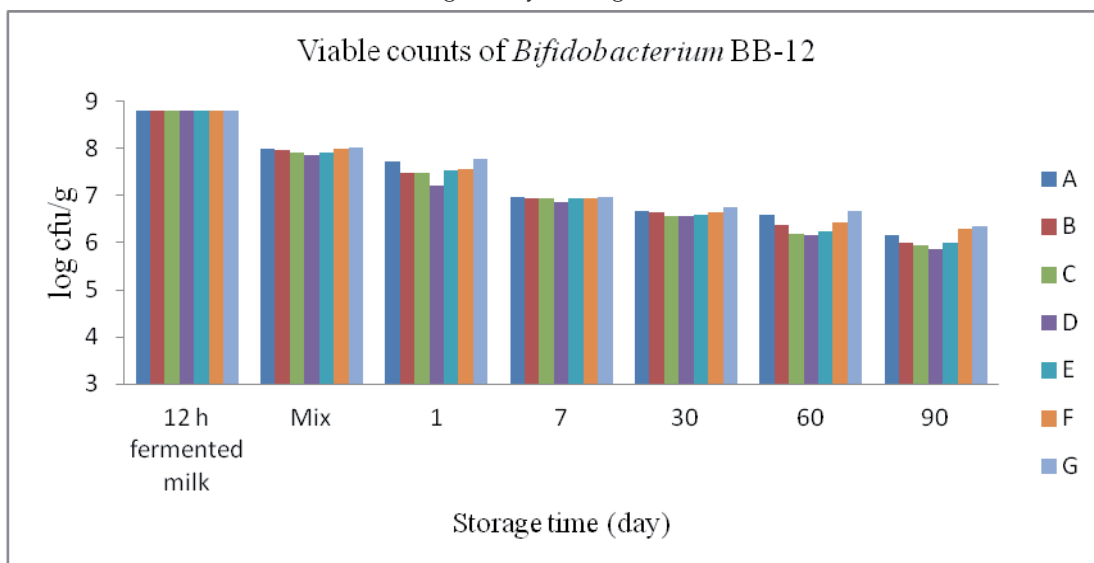


Figure 2. Viable counts of *Bifidobacterium* BB-12 in symbiotic ice-cream with different glucose oxidase and ascorbic acid concentrations during 90 days storage at  $-18\text{ }^{\circ}\text{C}$

*L. acidophilus* and *Bifidobacterium* BB-12 counts of fermented milks were 8.74 and 8.79 log CFU/g. During freezing, the viable counts of *L. acidophilus* and *Bifidobacterium* BB-12 decreased by 0.87-1.01 and 0.23-0.64 log unit, respectively. The decline in *L. acidophilus* counts, as a result of freezing, was expected due to the freeze injury of cells, leading eventually to the death of cells. On the other hand, reduction of bacteria in ice cream after mixing might be linked to the dilution of fermented milks since the addition of 10 % of fermented milk corresponded to a dilution of 10 times. This corresponded to a decrease in the viable cell count for 1 log CFU/g. 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 Akin et al. (2007), Akalin and Erisir (2008) and Ranadheera et al. (2013). The viable counts of *Bifidobacterium* BB-12 in frozen ice creams were found similar to mixes. Previous reports showed that oxygen toxicity caused by the incorporation of air during the ice cream production may seriously affect the growth of anaerobic bacteria (Homayouni et al., 2008; Ferraz et al., 2012). Such trends were not observed in the present study. Similarly, Ranadheera et al. (2013) and Da Silva et al. (2014) showed no oxygen effect on *B. lactis* survival in low overrun from goats' milk ice cream. The overrun values of ice creams were about 28 % in the present study. Glucose oxidase and ascorbic acid may act as an oxygen scavenger, thus the toxicity of oxygen might be prevented. Dave and Shah (1997) and Cruz et al. (2010) reported similar results in probiotic yoghurt. The effect of glucose oxidase and ascorbic acid addition on the viable probiotic bacteria counts was significant ( $p < 0.01$ ). The highest viable probiotic bacteria numbers were in samples with 0.1 % ascorbic acid (sample G). The addition of ascorbic acid improved the survival of *L. acidophilus* and *Bifidobacterium* BB-12. The viable counts of *L. acidophilus* and *B. lactis* increased with the level of ascorbic acid ( $p < 0.01$ ) most probably due to the possible oxygen scavenging effects of ascorbic acid. Dave and Shah (1997) reported similar results in probiotic yoghurt. Addition of glucose negatively affected the viable counts of probiotic bacteria which could be related to an increasing content of hydrogen peroxide in the ice cream. Glucose oxidase is

oxidized by molecular oxygen ( $O_2$ ) which is then reduced to hydrogen peroxide ( $H_2O_2$ ) (Raba and Mottola, 1995) that would cause an inhibitory effect on probiotic bacteria. However, it also produces oxygen, which can be incorporated throughout the storage, and therefore may be considered as a disadvantage. With the increase in glucose oxidase content, viable counts of probiotic bacteria slightly decreased ( $p < 0.01$ ). Cruz et al. (2012) reported similar results.

Viable counts of *L. acidophilus* and *Bifidobacterium* BB-12 decreased in all samples during 90 days storage period ( $p < 0.01$ ). However, the observed decrease was lower in samples supplemented with ascorbic acid in comparison to the control and the samples supplemented with glucose oxidase. The viable counts at the end of 90 days storage period was 106 CFU/g in the control (A) and the samples supplemented with ascorbic acid (E, F, G). Akin et al. (2007), Akalin and Erisir (2008), Ranadheera et al. (2013) and Da Silva et al. (2014) reported that the numbers of probiotic bacteria decreased during storage.

*L. acidophilus* counts of probiotic cheeses, Petit Suisse cheese and fermented dairy beverages were reported between 9.11-9.42 log CFU/g (Gomes et al., 2011), above 7.7 log CFU/g (Esmerino et al., 2013) and between 8.68-8.83 log CFU/g (Castro et al., 2013a, b), respectively. Gomes et al. (2011) reported that *Bifidobacterium animalis* counts in probiotic cheeses were between 8.31-8.96 log CFU/g. Esmerino et al. (2013) and (Castro et al., 2013a) reported that *Bifidobacterium lactis* counts in Petit Suisse cheese and fermented milks were above 7 log CFU/g and between 3-4.54 log CFU/g, respectively. Ice cream samples produced in the present study contained *L. acidophilus* and *Bifidobacterium* BB-12 at counts above 6 billion probiotic cells/g which was lower than the values found in fermented milks and yogurts (10 billion cells per portion). It is more practical to ingest a volume of 200 mL of fermented milk or yogurt compared with consuming a portion of ice cream, so the consumer preference towards fermented milks or yogurts as food matrices to deliver probiotic bacteria was higher. However, our findings confirm the ice cream might be used as a carrier of probiotic bacteria, since all samples (except control and contain 0.1 % glucose oxidase) reached the minimal value ( $10^6$  CFU/g) capable of delivering

Table 1. Some chemical properties of symbiotic ice creams (n=2)\*\*

Ice cream sample*	pH	Titrateable acidity (%L.A)	Dry matter (%)
A	6.19±0.01 <sup>a</sup>	0.26±0.00 <sup>a</sup>	34.56±0.39 <sup>a</sup>
B	6.20±0.16 <sup>a</sup>	0.26±0.01 <sup>a</sup>	34.56±0.35 <sup>a</sup>
C	6.21±0.17 <sup>a</sup>	0.27±0.01 <sup>a</sup>	34.75±0.18 <sup>a</sup>
D	6.21±0.16 <sup>a</sup>	0.27±0.02 <sup>a</sup>	34.81±0.09 <sup>a</sup>
E	6.21±0.17 <sup>a</sup>	0.29±0.01 <sup>a</sup>	34.92±0.27 <sup>a</sup>
F	6.20±0.15 <sup>a</sup>	0.28±0.01 <sup>a</sup>	35.30±0.024 <sup>a</sup>
G	6.16±0.17 <sup>a</sup>	0.30±0.01 <sup>a</sup>	34.48±0.38 <sup>a</sup>

\*A: Control, B: %0.025, C: %0.050, D: %0.1 contain glucose oxidase, E: %0.025, F: %0.050, G: %0.1 contain ascorbic acid  
 \*\*a,b,c: Means in the same column followed by different letters were significantly different (p<0.01)

Table 2. Physical properties of symbiotic ice- creams (n=2)\*\*

Ice creams*	Viscosity	Overrun	First	Complete	Melting rate	Melting rate
	(cp)	(%)	dripping times (s)	melting times (s)	at 30 min (%)	at 60 min (%)
A	6819±29 <sup>a</sup>	28.38±0.08 <sup>a</sup>	1265±18 <sup>a</sup>	4020±180 <sup>a</sup>	66.76±1.28 <sup>a</sup>	86.44±1.11 <sup>a</sup>
B	6628±192 <sup>a</sup>	28.79±0.39 <sup>a</sup>	1269.5±19.5 <sup>a</sup>	3808.5±58.5 <sup>a</sup>	65.905±1.58 <sup>a</sup>	88.625±0.71 <sup>a</sup>
C	6553±183 <sup>a</sup>	28.83±0.225 <sup>a</sup>	1261±14 <sup>a</sup>	3783±42 <sup>a</sup>	62.98±1.52 <sup>a</sup>	87.44±1.02 <sup>a</sup>
D	6616±176 <sup>a</sup>	28.27±0.10 <sup>a</sup>	1242.5±1.5 <sup>a</sup>	3770.5±38.5 <sup>a</sup>	69.19±1.16 <sup>a</sup>	84.7±0.96 <sup>a</sup>
E	7118±214 <sup>a</sup>	28.68±0.145 <sup>a</sup>	1338.5±47.5 <sup>a</sup>	4015.5±142.5 <sup>a</sup>	64.74±1.04 <sup>a</sup>	84.195±1.58 <sup>a</sup>
F	6972±88 <sup>a</sup>	28.29±0.06 <sup>a</sup>	1230.5±28.5 <sup>a</sup>	3691.5±85.5 <sup>a</sup>	63.41±1.37 <sup>a</sup>	89.66±0.65 <sup>a</sup>
G	7150±170 <sup>a</sup>	28.55±0.35 <sup>a</sup>	1217±17 <sup>a</sup>	3651±51 <sup>a</sup>	62.72±0.73 <sup>a</sup>	86.805±0.86 <sup>a</sup>

\*A: Control, B: %0.025, C: %0.050, D: %0.1 contain glucose oxidase, E: %0.025, F: %0.050, G: %0.1 contain ascorbic acid  
 \*\*a,b,c: Means in the same column followed by different letters were significantly different (p<0.01)

therapeutic benefits to the consumer at the end of 90 days storage. The Turkish Legislation establishes a minimum quantity of probiotic bacteria at least 6 log CFU/g (Anon, 2001).

#### Physico-chemical properties

Results considering chemical properties of ice-creams were presented in Table 1. The addition of glucose oxidase and ascorbic acid in different lev-

els had no significant influence (p>0.05) on the dry matter, pH and titrateable acidity of ice cream samples.

The physical properties of ice-creams were shown in Table 2. Examinations of the viscosity, overrun value, first dripping time, complete melting time and melting rates of symbiotic ice creams did not reveal any significant differences among the control, the ice creams supplemented with glucose oxidase

Table 3. Sensory evaluation of symbiotic ice-cream (n=2)\*\*

Ice creams*	Coldness	Firmness	Viscosity	Smoothness	Colour and appearance	Mouth coating	Taste and odour
A	6.11±0.34 <sup>a</sup>	7.75±0.92 <sup>a</sup>	7.53±0.61 <sup>a</sup>	8.83±0.29 <sup>a</sup>	9.53±0.15 <sup>a</sup>	8.21±0.36 <sup>a</sup>	8.81±0.19 <sup>a</sup>
B	6.14±0.53 <sup>a</sup>	6.59±0.42 <sup>a</sup>	7.31±1.08 <sup>a</sup>	8.16±0.85 <sup>a</sup>	9.17±0.39 <sup>a</sup>	7.63±0.48 <sup>a</sup>	8.32±0.01 <sup>a</sup>
C	5.49±0.73 <sup>a</sup>	7.21±0.46 <sup>a</sup>	7.68±0.83 <sup>a</sup>	8.99±0.74 <sup>a</sup>	9.28±0.28 <sup>a</sup>	8.24±0.32 <sup>a</sup>	8.26±0.41 <sup>a</sup>
D	5.59±0.97 <sup>a</sup>	7.46±0.88 <sup>a</sup>	7.48±0.65 <sup>a</sup>	7.79±0.09 <sup>a</sup>	9.00±0.00 <sup>a</sup>	7.84±0.38 <sup>a</sup>	7.68±0.22 <sup>a</sup>
E	5.82±0.97 <sup>a</sup>	7.1±1.01 <sup>a</sup>	7.28±0.86 <sup>a</sup>	8.33±0.56 <sup>a</sup>	9.19±0.03 <sup>a</sup>	8.19±1.04 <sup>a</sup>	7.88±0.42 <sup>a</sup>
F	5.4±0.71 <sup>a</sup>	6.97±0.7 <sup>a</sup>	7.59±0.67 <sup>a</sup>	8.59±0.74 <sup>a</sup>	9.02±0.32 <sup>a</sup>	7.86±0.71 <sup>a</sup>	7.98±0.52 <sup>a</sup>
G	5.04±0.19 <sup>a</sup>	7.37±1.01 <sup>a</sup>	7.69±0.69 <sup>a</sup>	8.79±0.64 <sup>a</sup>	8.76±0.46 <sup>a</sup>	7.86±0.36 <sup>a</sup>	8.4±0.3 <sup>a</sup>

\*A: Control, B: %0.025, C: %0.050, D: %0.1 contain glucose oxidase, E: %0.025, F: %0.050, G: %0.1 contain ascorbic acid  
 \*\*a,b,c: Means in the same column followed by different letters were significantly different (p<0.01)

and ascorbic acid. Also, increased levels of glucose oxidase and ascorbic acid had insignificant effect on the above mentioned physical properties of symbiotic ice creams (p>0.05). Despite the statistical insignificance, the melting properties of ice creams supplemented with ascorbic acid showed better results than ice cream samples supplemented with glucose oxidase, which might be related to the physical structure of ice cream. The three main structural components of ice cream are air cells, fat globules and ice crystals (Ranadheera et al., 2013). During the storage of ice cream, a number of changes in the physical structure of the product may potentially occur such as disproportionation and coalescence of air cells (Sofjan and Hartel, 2004) and ice recrystallization, whereby small ice crystals melt and large crystals grow simultaneously (Akalın and Erisir, 2008). Small crystals, with a slightly lower melting point, are more sensitive to temperature fluctuations than large crystals (Akalın and Erisir, 2008). Thus this phenomenon would be likely to affect the melting properties of the symbiotic ice creams.

#### Sensory evaluations

Addition of glucose oxidase and ascorbic acid had no significant effect on the tested sensory characteristics (Table 3) of symbiotic ice creams. Neither did increased levels of glucose oxidase and ascorbic acid have a significant effect on the sensory properties of symbiotic ice creams (p>0.05). A yogurt or probiotic flavour was not found to be particularly noticeable which might rely on the high pH of the ice-cream. All of the samples were characterised by a good total impression, were evaluated as medium sour and did not have any marked off-flavour.

#### Conclusions

Effects of different glucose oxidase and ascorbic acid levels on physical and sensory characteristics of symbiotic ice-cream were investigated. Both, ascorbic acid and glucose oxidase concentration had no effect on physical and sensory properties of ice cream.

Survival of probiotic bacteria was also studied in symbiotic ice-cream with different glucose oxidase and ascorbic acid concentrations during 90 days. Addition of glucose oxidase and ascorbic acid significantly (p<0.01) affected the viable counts of probiotic bacteria strains. The highest viable count was found in samples with 0.1 % ascorbic acid. Increasing ascorbic acid concentration stimulated the growth of *L. acidophilus* and *Bifidobacterium* BB-12 which could be due to oxygen scavenging effects of

ascorbic acid. On contrary, increasing glucose oxidase concentration had negative effect on the growth of *L. acidophilus* and *Bifidobacterium* BB-12, probably due to formation of hydrogen peroxide. The obtained results suggested that the addition of ascorbic acid stimulated the growth of *L. acidophilus* and *Bifidobacterium* BB-12 and could be recommended for ice cream production.

### Acknowledgements

This study was financially supported by the HUBAK (Project No: 13117) and extracted from the Master thesis of Feride Dasnik.

*Utjecaj različitih koncentracija askorbinske kiseline i glukoza oksidaze na preživljavanje probiotičkih bakterija i na fizikalna i senzorska svojstva u simbiotičkom sladoledu*

### Sažetak

U ovom je istraživanju ispitivan utjecaj dodatka različitih količina askorbinske kiseline i glukoza oksidaze na svojstva simbiotičkog sladoleda. Sladoled koji je sadržavao inulin (2 % (w/w)) proizveden je miješanjem obogaćenog mlijeka fermentiranog probiotičkim sojevima s mješavinom sladoleda koja je sadržavala askorbinsku kiselinu i različite koncentracije glukoza oksidaze (0,025, 0,05, 0,1 (w/w)). Kulture su uzgajane (37 °C, 12 h) u obranom mlijeku obrađenom UHT sterilizacijom. Fermentirano mlijeko dodavano je u sladolednu smjesu u količini od 10 % w/w. Povećanje koncentracije askorbinske kiseline stimuliralo je rast sojeva *Lactobacillus acidophilus* LA-5 (*L. acidophilus*) i *Bifidobacterium animalis* subsp. *lactis* BB-12 (*Bifidobacterium* BB-12), dok je povećanje koncentracije glukoza oksidaze negativno utjecalo na rast istih sojeva. Međutim, ni askorbinska kiselina niti glukoza oksidaza nisu utjecale na fizikalna i senzorska svojstva sladoleda. Rezultati su pokazali da dodatak askorbinske kiseline stimulira rast *L. acidophilus* i *Bifidobacterium* BB-12 te se stoga može preporučiti kao dodatak u proizvodnji sladoleda.

*Ključne riječi:* sladoled, probiotička bakterija, askorbinska kiselina, glukoza oksidaza

### References

1. Aime, DB., Arntfield, SD., Malcolmson, LJ., Ryland, D. (2001): Textural analysis of fat reduced vanilla ice cream products, *Food Research International* 34, 237-246.  
doi: dx.doi.org/10.1016/S0963-9969(00)00160-5
2. Akalin, AS., 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 (4), M184-M188.  
doi: dx.doi.org/10.1111/j.1750-3841.2008.00728.x
3. Akin, M.B., Akin, MS., Kirmaci, 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 (1), 93-99.  
doi: dx.doi.org/10.1016/j.foodchem.2006.11.030
4. Anonymous, 2001. Fermente Sütler Tebliği (Fermented Milk Notification). Turk Gıda Kodeksi No: 2001/21, Page 1-6 Ankara.
5. Castro, W.F., Cruz, AG., Rodrigues, D., Ghiselli, G., Oliveira, CAF., Faria, JAF., Godoy, HT. (2013a). Effects of different whey concentrations on physicochemical characteristics and viable counts of starter bacteria in dairy beverage supplemented with probiotics, *Journal of Dairy Science* 96 (1), 96-100.  
doi: dx.doi.org/10.3168/jds.2012-5576
6. Castro, WF., Cruz, AG., Bisinotto, MS., Guerreiro, LMR., Faria, JAF., Bolini, HMA., Cunha, RL., Deliza, R. (2013b). Development of probiotic dairy beverages: Rheological properties and application of mathematical models in sensory evaluation, *Journal of Dairy Science* 96 (1), 16-25.  
doi: dx.doi.org/10.3168/jds.2012-5590
7. Cruz, A.G., Faria, JAF., Van Dender, AGF. (2007). Packaging system and probiotic dairy foods, *Food Research International* 40, 951-995.  
doi: dx.doi.org/10.1016/j.foodres.2007.05.003
8. Cruz, A.G., Antunes, A.E.C., Sousa, ALOP, Faria, JAF., Saad, SMI. (2009). Ice-cream as a probiotic food carrier, *Food Research International* 42 (9), 1233-1239.  
doi: dx.doi.org/10.1016/j.foodres.2009.03.020
9. Cruz, A.G., Faria, JAF., Walter, EHM., Andrade, RRR., Cavalcanti, N., Oliveira, CAF., Granato, D. (2010): Optimization of the processing of probiotic yoghurt added with glucose oxidase using the response surface methodology, *Journal of Dairy Science* 93, 1058-1069.  
doi: dx.doi.org/10.3168/jds.2010-3336
10. Cruz, AG., Castro, WF., Faria, JAF., Bogusz, S., Granato, D., Celeguini, RMS. (2012). Glucose oxidase: A potential option to decrease the oxidative stress in stirred probiotic yogurt. *LWT - Food Science and Technology* 47, 512-515.  
doi: dx.doi.org/10.1016/j.lwt.2012.01.037



11. Cruz, A.G., Castro, WF., Faria, JAF., Lollo, PCB., Amaya-Farfan, J., Freitas, MQ., Rodrigues, D., Oliveira, CAF., Godoy, HT. (2012): Probiotic yoghurts manufactured with increased glucose oxidase levels: Post-acidification, proteolytic patterns, survival of probiotic microorganisms, production of organic acid and aroma compounds, *Journal of Dairy Science* 95, 2261-2269.  
doi: dx.doi.org/10.3168/jds.2011-4582
12. Cruz, AG., Castro, WF., Faria, JAF., Bolini, HMA., Celeghini, RMS., Raices, RSL., Oliveira, CAF., Freitas, MQ., Conte Júnior, CA., Mársico, ET. (2013): Stability of probiotic yogurt added with glucose oxidase: in plastic materials with different permeability oxygen rates during the refrigerated storage, *Food Research International* 51 (2), 723-728.  
doi: dx.doi.org/10.1016/j.foodres.2013.01.028
13. Da Silva, P.D.L., De Fatima Bezerra, M., Dos Santos, K.M.O., Correia, RTP. (2014): Potentially probiotic ice cream from goat's milk: Characterization and cell viability during processing, storage and simulated gastrointestinal conditions, *LWT-Food Science and Technology* (in press).
14. Dave, R.I., Shah, NP. (1996): Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *Bifidobacteria*, *Journal of Dairy Science* 79, 1529-1536.  
doi: dx.doi.org/10.3168/jds.S0022-0302(96)76513-X
15. Dave, R.I., Shah NP. (1997): Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of Probiotic bacteria in yoghurts made with commercial starter cultures. *International Dairy Journal* 7, 435-443.  
doi: dx.doi.org/10.1016/S0958-6946(97)00026-5
16. Esmerino, E.A., Cruz, AG., Pereira, EPR., Rodrigues, JB., Faria, JAF., Bolini, HMA. (2013): The influence of sweeteners in probiotic Petit Suisse cheese in concentrations equivalent to that of sucrose, *Journal of Dairy Science* 96 (9), 5512-5521.  
doi: dx.doi.org/10.3168/jds.2013-6616
17. Ferraz, JL., Cruz, AG., Cadena, RS., Freitas, MQ., Pinto, UM., Carvalho, CC., Faria, JAF., H. Bolini, MA. (2012): Sensory acceptance and survival of probiotic bacteria in ice cream produced with different overrun levels, *Journal of Food Science* 77, S24-S28.  
doi: dx.doi.org/10.1111/j.1750-3841.2011.02508.x
18. Güler-Akin, M.B., Akin, MS. (2007): Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk, *Food Chemistry* 100 (2), 788-793.  
doi: dx.doi.org/10.1016/j.foodchem.2005.10.038
19. Güven, M., Karaca, O.B. (2002): The effects of varying sugar content and fruit concentration on the physical properties of vanilla and fruit ice-cream-type frozen yogurts, *International Journal of Dairy Technology* 55, 27-31.  
doi: dx.doi.org/10.1046/j.1471-0307.2002.00034.x
20. Gomes, A.A., Braga, SP., Cruz, AG., Cadena, RS., Lollo, P.C.B., Carvalho, C., Amaya-Farfan, J., Faria, JAF., Bolini, H.M.A. (2011): Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physicochemical features and sensory performance compared with commercial cheeses, *Journal of Dairy Science* 94 (10), 4777-4786.  
doi: dx.doi.org/10.3168/jds.2011-4175
21. Homayouni, A., Azizi, A., Ehsani, M.R., Yarmand, M.S., Razavi, S.H. (2008): Effect of microencapsulation and resistant starch on probiotic survival and sensory properties of synbiotic ice cream, *Food Chemistry* 111, 50-55.  
doi: dx.doi.org/10.1016/j.foodchem.2008.03.036
22. Lollo, PCB., Cruz, AG., Morato, PN., Moura, CS., Carvalho-Silva, LB., Oliveira, CAF., Faria, JAF., Amaya-Farfan, J. (2012): Probiotic cheese attenuates exercise-induced immune suppression in Wistar rats, *Journal of Dairy Science* 95 (7), 3549-3558.  
doi: dx.doi.org/10.3168/jds.2011-5124
23. Raba, J., Mottola, H.A. (1995): Glucose oxidase as an Analytical reagent, *Critical Reviews in Analytical Chemistry* 25 (1), 1-42.  
doi: dx.doi.org/10.1080/10408349508050556
24. Ranadheera, CS., Evans, C.A., Adams, MC., Baines, SK. (2013): Production of probiotic ice cream from goat's milk and effect of packaging materials on product quality, *Small Ruminant Research* 112, 174-180.  
doi: dx.doi.org/10.1016/j.smallrumres.2012.12.020
25. Rodrigues, D., Rocha-Santos, T., Sousa, S., Gomes, AM., Pin-tado, M.M., Malcata, FX., Sousa Lobo, J.M., Freitas, A.C. (2011): On the viability of five probiotic strains when immobilised on various polymers, *International Journal of Dairy Technology* 64, 137-144.  
doi: dx.doi.org/10.1111/j.1471-0307.2010.00627.x
26. Sofjan, R.P., Hartel, R.W. (2004): Effects of overrun on structural and physical characteristics of ice cream, *International Dairy Journal*, 14 (3), 255-262.  
doi: dx.doi.org/10.1016/j.idairyj.2003.08.005
27. T.S.E. (1989): Türk Standartları Enstitüsü. Yogurt Standardı. T.S. 1330, Page 1-10 Ankara.