INT, J, BIOL. BIOTECH., 15 (1): 63-69, 2018.

EFFECT OF DIFFERENT AMOUNTS OF SUGAR AND FAT ON THE VIABILITY OF *LACTOBACILLUS CASEI*, PHYSICAL, CHEMICAL AND SENSORY PROPERTIES OF PROBIOTIC ICE CREAM

Azita Shahsavan, Rezvan Pourahmad* and Peyman Rajaei

Department of Food Science and Technology, College of Agriculture, Varamin-Pishva Branch, ***Islamic Azad University****, Varamin, Iran*
*Corresponding Author: rjpourahmad@yahoo.com, rezvanpourahmad@iauvaramin.ac.ir

ABSTRACT

Using ice cream as probiotic's food carrier can affect gut health and prevention of diseases especially children who are the special consumers of this product. The purpose of this study was to evaluate the effect of sugar and fat on survival of *Lactobacillus casei*, physical, chemical and sensory properties of probiotic ice cream. Nine ice cream formulations with different amounts of fat (5, 7.5 and 10 %) and sugar (14, 16 and 18 %) were produced. *Lactobacillus casei* (10^8 CFU/mL) was inoculated to the samples before freezing. The results showed that by increasing the amount of sugar and fat, the viability of *Lactobacillus casei* and over run in ice cream samples significantly decreased ($p < 0.05$) but dry matter, acidity and melting resistance significantly increased ($p < 0.05$). During storage, the number of probiotic bacteria significantly decreased ($p < 0.05$) but acidity significantly increased ($p < 0.05$).The sample containing 16% sugar and 5% fat had the highest probiotic bacteria population. The sample containing 16% sugar and 7.5% fat had the highest overall acceptance and according to this point that probiotic bacteria population in this sample was higher than the recommended by the International Dairy Federation (10^6 CFU/mL) , as a result this sample was introduced as the best treatment.

Key-words: Ice cream, *Lactobacillus casei*, Probiotic

INTRODUCTION

Ice cream is a frozen mixture of milk, sweetener, stabilizer, emulsifier, flavor and aroma compounds. Other constituents including egg products, coloring, and starch hydrolyzed products may be added. Three main structural compounds are air cells, ice crystals and fat globules being dispersed in a continuous phase of a non- frozen solution (Muse and Hartel, 2004). Ice cream as a multiphase complex system consists of a concentrated frozen matrix containing ice crystals, air cells and fat globules. Ice cream is a good source of essential amino acids of milk proteins, vitamins, and minerals (Kailasapathy and Sultana, 2003). Fat plays an important role in developing flavor and a solid structure during freezing so it has consistency, appearance and resistance to melting. High fat content results in a dry and grainy texture and low fat content develops a smooth, uniform and partly slimy texture. Cream, butter, butter oil and vegetable oil may provide fat in the ice cream. Sugar (mainly sucrose) is needed to develop taste and lower the freezing point. Very low content of sugar causes to forming large amount of ice and a high sugar content results in an extra sweet ice cream. So sucrose is partly replaced by glucose syrup. It causes higher viscosity especially when a large part of water has been frozen (Hashemi *et al*., 2015).

Probiotics are microorganisms exerting healthful effects on the host if present living in sufficient number. The effects include inhibiting bacterial pathogens, lowering serum cholesterol level, reducing the incidence of constipation, diarrhea, and colon cancer, improving lactose digestion and calcium absorption, and stimulating immune system. Probiotic bacteria mainly include lactobacilli and bifidobacteria (Muse and Hartel, 2004; Sanchez, 2009). Among probiotic dairy products, probiotic ice cream and fermented frozen dessert are the favorite products (Hong and Marshall, 2001).

Functional foods contain components beyond traditional nutrients playing role in human health. One of methods for changing foods into functional products is use of probiotic bacteria (Kailasapathy and Sultana, 2003). Some studies have introduced ice cream as a suitable carrier of probiotics to the consumers (Hekmat and McMahon, 1992; Soccol *et al.*, 2010). Hashemi *et al*. (2015) investigated the influence of replacing 5% fat and sugar by inulin and lactulose, respectively, on the viability of *Bifidobacterium lactis* and physicochemical and sensory properties of ice cream. Total solids, pH, melting rate and organoleptic scores of low-fat and/or low-sugar ice cream samples did not differ significantly from the control sample. Overrun and hardness of low-fat samples were significantly higher than that of the control sample. The population of *B. lactis* in the low-sugar synbiotic ice creams was significantly lower than that of other samples at the end of 90-day storage. Homayouni *et al*., (2008) studied the increased viability of probiotic bacteria by encapsulation in traditional synbiotic ice cream contaninig 1% resistant starch. Also they examined the viability of probiotic bacteria in forms of free and encapsulated over six- month storage at -20ºC. Encapsulation of *Lactobacillus casei* and *Bifidobacterium lactis* in beads made- form calcium alginate and resistant starch caused a 30% increase in viability of bacteria. Other researchers studied the surviral of *L. delbrueckii* was not significantly affected by the formulation. In fact, reduced fat content did not increase the viability of *L. delbrueckii* (Santos Leandro *et al.*, 2013).

Although the viability of some probiotic bacteria in some frozen dessert has been studied, few studies have been conducted on the production of non- fermented probiotic ice cream. Since sugar and fat used in ice cream may resulted in obesity and its undesirable effects on the consumers especially children, these harmful effect can be diminished by reducing the amount of sugar and fat. Thus, the objective of this study was to investigate the effect of different concentrations of sugar and fat on viability of *L.casei* and the physicochemical and sensory properties of probiotic ice cream during storage.

MATERIALS AND METHODS

Materials

The materials included skim milk (Choopan Co., Iran), sugar (Karaj sugar Co.), cream (Taravat Co., Iran), skim milk powder (Pak Dairy Co., Iran), stabilizer and emulsifier (Palsgaard, Denmark).

Preparation of starter culture

Lactobacillus casei DVS starter was purchased from CHR HASNSEN Co. (Denmark). According to manufacturer’s instructions, starter package was added to 1000 mL sterilized milk and stirred thoroughly to solve all starter granules.

Preparation of probiotic ice cream

First skim milk and cream (40% fat) were mildly heated in the formulation tank. When the temperature reached to 50ºC, skim milk powder, stabilizer and sugar were added. Then flavor compound (vanillin) was added. The prepared mixture was pasteurized at 72ºC for 15 min. Then, the ice cream mixture was homogenized in a two- step homogenizer at 2500 and 500 Psi. Then it cooled at 4ºC and kept at this temperature for 12 h. *L. casei* (10^8 CFU/mL) was added to the ice cream mixture and immediately frozen, packed in cups and transferred to -24ºC fridge and stored for 90d. The treatments of study are presented in Table 1.

Table 1. The treatments of study

Treatments	Composition
S_1F_1	14% sugar and 5% fat
S_1F_2	14% sugar and 7.5% fat
S_1F_3	14% sugar and 10% fat
S_2F_1	16% sugar and 5% fat
S_2F_2	16% sugar and 7.5% fat
S_2F_3	16% sugar and 10% fat
S_3F_1	18% sugar and 5% fat
S_3F_2	18% sugar and 7.5% fat
S_3F_3	18% sugar and 10% fat

Lactobacillus casei count

MRS agar (Merck, Germany) was used for enumeration of probiotic bacteria. The plates were incubated anaerobically at 37°C for at least 72 h (Tharmaraj, 2003).

Physical tests

Over run was calculated by comparing a certain volume of ice cream with the same volume before freezing (Akalin *et al*., 2007). To calculate melting resistance, first 30 g of ice cream were placed on a mesh with 2 mm pores on top of a glass funnel before freezing (Manning and Gibson, 2004). Then the sample was incubated at 25ºC for 45 min. The amount of melted ice cream in the erlene was considered as the indicator of melting quality (Sun-Waterhouse *et al*., 2013).

Chemical tests

Acidity and dry matter were measured by AOAc method (AOAC, 2005).

Sensory evaluation

Sensory properties including flavor, texture, color and total acceptance were evaluated by 10 trained panelists. 5- point hedonic scale was used. Scores 5, 4, 3, 2 and 1 represented very good, good, intermediate, bad and very bad, respectively (Haynes and Playne, 2002).

Statistical analysis

Nine treatments with three replications were studied. Data were analyzed by variance analysis and Duncan test using SPSS software.

RESULTS AND DISCUSSION

***Lactobacillus casei* count**

Number of *Lactobacillus casei* in ice cream samples is shown in Table 2. The lowest number of *L.casei* was observed for sample S_1F_3 (14% sugar and 10% fat).The highest number of *L.casei* was found for sample S_2F_1 (16% sugar and 5% fat). Sugar and fat content had significant ($p < 0.05$) effect on the viability of *L.casei*. Number of *L.casei* in probiotic ice cream samples significantly ($p < 0.05$) decreased over time. The reasons seem to be formation and growth of ice crystals, physical force when mixing, increased osmotic pressure caused by transformation of water into ice and pH decrease which affect the viability of lactobacilli (Marshal *et al*., 2012). Factors including low acidity, high fat and non- fat solids content as well as casein, sucrose and lactose which may exert protective effect on the bacteria are among effective factors influencing the viability of the bacteria (De Angelis and Gobbetti, 2004). Some researchers studied the viability of *L. acidophilus* and *L.casei* in probiotic ice cream. Their results revealed that encapsulation of probiotics with 1, 2 and 3% sodium alginate could increase their viability up 95.25, 99.06 and 99.11% showing no significant ($p > 0.05$) difference from control sample. Viability of probiotics during storage at -20ºC increased (Songtummin and Leenanon, 2016). Their results are different from our findings and the reason may be lack of bacteria coating in probiotic ice cream.

Table 2. *Lactobacillus casei* count (log CFU/mL) in probiotic ice cream samples (SD ± Mean).

Sample	Upon Production	30 d	60 d	90 d
S_1F_1	$8.94±0.03^{Aab}$	$7.80±0.01^{Bcd}$	$6.80±0.01^{Cd}$	$6.65±0.01^{Dd}$
S_1F_2	$8.87±0.02^{Ac}$	$7.78±0.02^{Be}$	$6.77±0.03^{Ce}$	$6.51±0.01^{Df}$
S_1F_3	$8.80±0.01^{Ae}$	$7.61±0.01^{Bf}$	$6.54±0.01^{Ch}$	$6.23±0.01^{Dh}$
S_2F_1	$8.95±0.01^{Aa}$	$7.93±0.01^{Ba}$	$7.76±0.01^{Ca}$	$7.38±0.01^{Da}$
S_2F_2	$8.95±0.00^{Aa}$	$7.93±0.00^{Ba}$	$6.86±0.01^{Cb}$	$6.74±0.01^{Dc}$
S_2F_3	$8.83±0.01^{Ade}$	$7.81±0.00^{Bc}$	$6.79±0.01^{Cd}$	$6.55±0.01^{De}$
S_3F_1	$8.95±0.03^{Aa}$	$7.92±0.00^{Ba}$	$6.64±0.00^{Cg}$	$6.85±0.00^{Db}$
S_3F_2	$8.91±0.05^{Ab}$	$7.85±0.01^{Bb}$	$6.82±0.00^{Cc}$	$6.64±0.01^{Dd}$
S_3F_3	$8.84±0.04^{Acb}$	$7.79±0.00^{Bde}$	$6.69±0.00^{Cf}$	$6.34±0.01^{Dg}$

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

Physical properties

Physical properties (over run and melting resistance) of probiotic ice cream samples are presented in Tables 3 and 4.

Table 3. Over run (%) of probiotic ice cream samples (SD ± Mean).

Sample	Upon Production	30 d	60 d	90 d
S_1F_1	60.32±0.57^{Aa}	60.31±0.76^{ABa}	60.30±0.50^{ABa}	60.29±0.28^{Ba}
S_1F_2	56.61±0.57^{Cb}	56.66±1.04^{Ab}	56.65±0.57^{Ab}	56.63±0.28^{Bb}
S_1F_3	54.62±0.57^{Bc}	54.63±0.57^{ABc}	54.65±1.00^{Ac}	54.64±0.57^{ABc}
S_2F_1	50.31±0.57^{ABd}	50.33±0.86^{Ad}	50.32±0.57^{ABd}	50.30±0.50^{Bd}
S_2F_2	48.00±1.00^{ABe}	48.01±0.57^{Ae}	47.99±0.57^{ABe}	47.98±0.28^{Be}
S_2F_3	45.33±0.57^{Af}	45.32±0.76^{Af}	45.32±0.57^{Af}	45.31±0.50^{Af}
S_3F_1	40.03±1.00^{Ag}	40.01±0.76^{Bg}	39.99±0.57^{Cg}	39.95±0.28^{Dg}
S_3F_2	37.34±0.57^{Ah}	37.33±0.28^{Ah}	37.32±0.28^{Ah}	37.30±0.28^{Ah}
S_3F_3	34.32±0.57^{Ai}	34.33±0.50^{Ai}	34.32±0.50^{Ai}	34.31±0.28^{Ai}

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

As shown in Table 3, the lowest over run was found for sample S_3F_3 (18% sugar and 10% fat). The highest over run was found for sample S_1F_1 (14% sugar and 5% fat). Indeed, over run showed a significant ($p<0.05$) decrease as the amount of sugar and fat increased. Storage time had no significant ($p<0.05$) effect on the over run. Considering the formulation and procedure of the probiotic ice cream, decrease in over run with increasing sugar and fat content is expected. In a similar study conducted by Hashemi *et al*. (2015) it was revealed that use of inulin instead of vegetable oil in synbiotic ice cream resulted in significant increase in over run compared to control sample. Also other researchers found that over run was related to fat content reversely as over run in ice cream containing 5% fat was more than 10% fat (Turgut and Cakmakci, 2009). Our results are in agreeemt with these findings.

Table 4. Rate of melting resistance (%) of probiotic ice cream samples (SD ± Mean).

Sample	Upon Production	30 d	60 d	90 d
S_1F_1	24.40±0.02^{ABg}	24.60±0.01^{Ah}	24.10±0.02^{Bi}	24.00±0.00^{Bf}
S_1F_2	24.50±0.02^{Ag}	24.90±0.01^{Ag}	24.90±0.01^{Ah}	24.60±0.02^{Aef}
S_1F_3	25.01±0.02^{Cf}	25.80±0.02^{Af}	25.10±0.01^{Bg}	25.03±0.01^{Cde}
S_2F_1	25.42±0.02^{Ae}	25.38±0.01^{Be}	25.39±0.01^{Bf}	25.41±0.01^{Acde}
S_2F_2	25.90±0.02^{Ad}	25.80±0.01^{Ae}	25.70±0.01^{Ae}	25.80±0.01^{Abcd}
S_2F_3	26.20±0.02^{Ac}	26.00±0.01^{Ad}	26.00±0.01^{Ad}	26.10±0.01^{Abc}
S_3F_1	26.30±0.02^{Bbc}	26.32±0.02^{Bc}	26.31±0.01^{Bc}	24.34±0.01^{Aab}
S_3F_2	26.50±0.01^{Bb}	26.55±0.01^{Bb}	26.50±0.01^{Bb}	26.63±0.01^{Aab}
S_3F_3	27.00±0.17^{Ca}	27.01±0.01^{Ca}	27.5±0.01^{Ba}	27.08±0.01^{Aa}

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

As shown in Table 4, the lowest melting resistance was observed for sample S_1F_1 (14% sugar and 5% fat). As the amount of sugar and fat increased, rate of melting decreased and melting resistance increased ($p<0.05$). Melting resistance showed no significant ($p>0.05$) difference over time. Akalin *et al*. (2007) investigated the effect of different levels of inulin and sugar on the rate of ice cream melting and obtained similar results.

Chemical properties

Chemical properties (acidity and dry matter) of probiotic ice cream samples are shown in Tables 5 and 6.

Table 5. Acidity (% lactic acid) of probiotic ice cream samples (SD ± Mean).

Sample	Upon Production	30 d	60 d	90 d
S_1F_1	$0.134{\pm}0.001^{Cc}$	$0.132{\pm}0.001^{Df}$	$0.137{\pm}0.000^{Bh}$	$0.137{\pm}0.000^{Af}$
S_1F_2	$0.148{\pm}0.001^{Cb}$	$0.147{\pm}0.001^{Dbc}$	$0.151{\pm}0.000^{Be}$	$0.152{\pm}0.000^{Ad}$
S_1F_3	$0.154{\pm}0.001^{Ca}$	$0.155{\pm}0.001^{Ba}$	$0.158{\pm}0.000^{Bb}$	$0.161{\pm}0.000^{Ab}$
S_2F_1	$0.134{\pm}0.002^{Cc}$	$0.137{\pm}0.002^{Bef}$	$0.138{\pm}0.000^{Ag}$	$0.138{\pm}0.001^{Ac}$
S_2F_2	$0.145{\pm}0.002^{Db}$	$0.150{\pm}0.006^{Cbc}$	$0.153{\pm}0.000^{Bd}$	$0.156{\pm}0.001^{Ac}$
S_2F_3	$0.153{\pm}0.001^{Ca}$	$0.153{\pm}0.001^{Cab}$	$0.157{\pm}0.000^{Bb}$	$0.158{\pm}0.000^{Ab}$
S_3F_1	$0.134{\pm}0.001^{Dc}$	$0.144{\pm}0.005^{Acd}$	$0.138{\pm}0.000^{Cg}$	$0.143{\pm}0.001^{Be}$
S_3F_2	$0.147{\pm}0.001^{Cb}$	$0.140{\pm}0.006^{Dde}$	$0.148{\pm}0.000^{Bf}$	$0.153{\pm}0.001^{Ad}$
S_3F_3	$0.152{\pm}0.002^{Ca}$	$0.153{\pm}0.001^{Ba}$	$0.155{\pm}0.000^{Bc}$	$0.157{\pm}0.001^{Abc}$

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

As shown in Table 5, the highest acidity was found for sample S_1F_3 (14% sugar and 10% fat) being significantly ($p < 0.05$) different from samples S_2F_3 and S_3F_3. The lowest acidity was observed for samples S_1F_1, S_2F_1 and S_3F_3 being significantly ($p < 0.05$) different from other samples. On the 30th day, the highest acidity was observed for sample S_1F_3 (14% sugar and 10% fat) and sample S_1F_1 (14% sugar, 5% fat) had the lowest acidity. On the 60th day, the highest and the lowest acidity were found for S_1F_3 (14% sugar and 10% fat) and S_1F_1(14% sugar and 5% fat), respectively. On the 90th day, the highest and the lowest acidity were observed for S_1F_3 (14% sugar and 10% fat) and S_1F_1 (14% sugar and 5% fat), being significantly ($p < 0.05$) difference over time due to growth and activity of probiotics and use of nutrients present in ice cream as well as production of organic acids. Manning and Gibson (2004) had reported similar results.

Table 6. Dry matter (%) of probiotic ice cream samples (SD ± Mean).

Sample	Upon Production	30 d	60 D	90 d
S_1F_1	$31.34{\pm}0.01^{Bi}$	$31.35{\pm}0.06^{ABi}$	$31.36{\pm}0.02^{ABi}$	$31.37{\pm}0.01^{Ai}$
S_1F_2	$33.43{\pm}0.01^{Ag}$	$33.44{\pm}0.05^{Ag}$	$33.45{\pm}0.02^{Ag}$	$33.46{\pm}0.00^{Ag}$
S_1F_3	$34.48{\pm}0.01^{Af}$	$34.49{\pm}0.05^{Af}$	$33.49{\pm}0.01^{Af}$	$34.50{\pm}0.00^{Af}$
S_2F_1	$33.22{\pm}0.00^{Ah}$	$33.23{\pm}0.01^{Ah}$	$35.23{\pm}0.01^{Ah}$	$33.24{\pm}0.01^{Ah}$
S_2F_2	$35.38{\pm}0.01^{Ad}$	$35.39{\pm}0.01^{Ad}$	$35.40{\pm}0.03^{Ad}$	$35.41{\pm}0.03^{Ad}$
S_2F_3	$36.95{\pm}0.000^{Ac}$	$35.95{\pm}0.01^{Ac}$	$36.96{\pm}0.02^{Ac}$	$36.97{\pm}0.01^{Ac}$
S_3F_1	$35.22{\pm}0.00^{Ae}$	$35.23{\pm}0.02^{Ae}$	$35.21{\pm}0.01^{Ae}$	$35.19{\pm}0.05^{Be}$
S_3F_2	$37.53{\pm}0.00^{Ab}$	$37.54{\pm}0.03^{Ab}$	$37.53{\pm}0.02^{Ab}$	$37.52{\pm}0.01^{Ab}$
S_3F_3	$38.91{\pm}0.01^{Ba}$	$38.92{\pm}0.01^{ABa}$	$38.93{\pm}0.03^{ABa}$	$38.94{\pm}0.02^{Aa}$

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

As shown in Table 6, the highest and the lowest dry matter content were observed for samples S_3F_3 (18% sugar and 10% fat) and S_1F_1 (14% sugar and 5% fat) respectively. Dry matter content increased significantly ($p<0.05$) as the amount of sugar and fat increased because of added sugar and fat into probiotic ice cream. Storage time had no significant ($p>0.05$) effect on dry matter content. Our results are in agreement with those of Hashemi *et al*. (2015).

Sensory properties

Sensory properties of probiotic ice cream samples are shown in Table 7. The effect of sugar, fat and storage time on the flavor of ice cream was significant ($p < 0.05$). The flavor score increased significantly ($p < 0.05$) with increasing sugar, fat and storage time. The amounts of sugar, fat and storage time had significant ($p < 0.05$) effect on the texture of ice cream. As sugar, fat, and storage time increased, the score of texture increased significantly ($p < 0.05$). The effect of sugar, fat and storage time on overall acceptance of ice cream was significant ($p < 0.05$). Upon production, the highest scores of flavor, texture, color and overall acceptance were observed for sample S_2F_2 (14% sugar and 7.5% fat) showing significant ($p < 0.05$) difference from other treatments except sample S_2F_3. On the 90th day, the highest score of flavor was found for sample S_2F_2 (16% sugar and 7.5% fat). The highest score of texture was belonged to sample S_3F_3 (18% sugar, 10% fat). The highest score of color was observed for samples S_1F_2, S_1F_3, S_2F_2, S_2F_3, S_3F_2 and S_3F_3. The highest score of overall acceptance was belonged to sample S_2F_2 (16% sugar, 7.5% fat) being significantly ($p < 0.05$) different from other treatments except sample S_2F_3. In a similar study, the effects of inulin and sugar on sensory properties of probiotic yogurt ice cream were investigated and the results showed that increased sugar content had no effect on sensory properties (Akalin *et al*., 2007). Similarly, Hashemi *et al*. (2015) studied sensory properties of low fat or low sugar synbiotic ice cream. To produce such product, 5% of vegetable fat or sugar was replaced with inulin or lactulose. Among sensory parameters only the scores of taste of synbiotic ice cream after 5 days of storage was significantly lower than that of control sample.

Table 7. Sensory properties of probiotic ice cream samples (SD ± Mean).

Sample	Flavor		Texture		Color		Overall acceptance	
	Upon production	90 d	Upon production	90 d	Upon production	90 D	Upon production	90 d
S_1F_1	$2.35±0.25^{Bh}$	$2.40±0.00^{Ai}$	$2.35±0.24^{Bh}$	$2.47±0.33^{Ag}$	$4.00±0.24^{Ac}$	$4.00±0.21^{Ab}$	$3.00±0.00^{Bg}$	$3.14±0.00^{Ah}$
S_1F_2	$2.62±0.00^{Bg}$	$2.68±0.42^{Ah}$	$3.00±0.00^{Bf}$	$3.03±0.21^{Af}$	$4.35±0.00^{Ab}$	$4.50±0.25^{Aa}$	$3.35±0.00^{Bg}$	$3.47±0.00^{AF}$
S_1F_3	$3.00±0.24^{Be}$	$3.11±0.21^{Ag}$	$3.70±0.24^{Bd}$	$3.75±0.25^{Ae}$	$4.50±0.00^{Aa}$	$4.50±0.00^{Aa}$	$3.48±0.00^{Bd}$	$3.59±0.00^{Ae}$
S_2F_1	$3.78±0.00^{Bb}$	$3.80±0.21^{Af}$	$2.80±0.25^{Bgi}$	$2.97±0.25^{Af}$	$4.00±0.00^{Ac}$	$4.00±0.00^{Ab}$	$3.56±0.00^{Bcd}$	$3.70±0.00^{Ad}$
S_2F_2	$4.03±0.24^{Ba}$	$4.19±0.00^{Ae}$	$2.35±0.24^{Bh}$	$3.96±0.21^{Ac}$	$4.50±0.00^{Aa}$	$4.50±0.21^{Aa}$	$4.32±0.00^{Ba}$	$4.36±0.00^{Aa}$
S_2F_3	$4.00±0.00^{Ba}$	$4.02±0.39^{Ad}$	$3.95±0.23^{Bb}$	$4.09±0.42^{Ab}$	$4.00±0.24^{Aa}$	$4.50±0.25^{Aa}$	$4.30±0.00^{Ba}$	$4.35±0.00^{Aa}$
S_3F_1	$2.81±0.00^{Bf}$	$2.87±0.21^{Ac}$	$3.15±0.24^{Ae}$	$2.98±0.42^{Bf}$	$4.00±0.00^{Ac}$	$4.00±0.00^{Ab}$	$3.20±0.00^{Bf}$	$3.28±0.00^{Ag}$
S_3F_2	$3.15±0.24^{Bd}$	$3.18±0.00^{Ab}$	$3.80±0.87^{Bc}$	$3.89±3.53^{Ad}$	$4.50±0.51^{Aa}$	$4.50±0.00^{Aa}$	$3.80±0.00^{Bb}$	$3.92±0.00^{Ab}$
S_3F_3	$3.47±0.47^{Bc}$	$3.51±0.39^{Aa}$	$4.20±0.25^{Be}$	$4.27±0.47^{Aa}$	$4.50±0.24^{Aa}$	$4.50±0.51^{Aa}$	$3.60±0.00^{Bc}$	$3.77±0.00^{Ac}$

Means with different capital letters in each row and means with different small letters in each column show significant different ($p < 0.05$).

CONCLUSION

As the amount of sugar and fat increased, viability of *L. casei* and over run of ice cream samples decreased significantly whereas dry matter, acidity, and melting resistance significantly increased ($p < 0.05$). Viability of *L. casei* and acidity of ice cream samples decreased and increased significantly ($p > 0.05$), respectively over storage. Also the effect of sugar and fat content as well as storage time on flavor, color, texture and total acceptance was significant ($p < 0.05$). The highest viability of *L. casei* was found for sample S_2F_1 (containing 16% sugar and 5% fat). Sample S_2F_2 (16% sugar and 7.5% fat) had the highest score of overall acceptance. Since the number of probiotic bacterium in this sample was higher than the limit recommended by International Dairy Federation (IDF) (10^6 CFU/mL), it was selected as the best treatment.

REFERENCES

Akalin, M.B., M.S. Akin and Z. Kirmaci (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.

AOAC (2005). Official methods of analysis, ice cream and frozen dessert, 18th editions. Association of Official Analytical Chemists, Washington, pp. 93-96.

De Angelis, M. and M. Gobbetti (2004). Environmental stress responses in *Lactobacillus*: A review. *Proteomics*, 4: 106-122.

Hashemi, M., H.R. Gheisari and S. Shekarforoush (2015). Preparation and evaluation of low-calorie functional ice cream containing inulin, lactulose and *Bifidobacterium lactis. International Journal of Dairy Technology*, 68 (2): 183-189.

Haynes, I.N. and M.J. Playne (2002). Survival of probiotic cultures in low-fat ice-cream. *Australian Journal of Dairy Technology*, 57(1): 10-14.

Hekmat, S. and D.J. McMahon (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Science*, 75(6): 1415-1422**.**

Homayouni, A., A. Azizi, M. R. Ehsani, M. S. Yarmand and S. H. Razavi (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, 11: 50-55.

Hong, S.H. and R.T. Marshall (2001). Natural exopolysaccharides enhance survival of lactic acid bacteria in frozen dairy desserts. *Journal of Dairy Science*, 84: 1367-1374.

Kailasapathy, K. and K. Sultana (2003). Survival and [beta]-D-galactosidase activity of encapsulated and free Lactobacillus acidophilus and Bifidobacterium lactis in ice-cream. *Australian Journal of Dairy Technology*, 58(3): 223-227.

Manning, T.S. and G.R. Gibson (2004). Prebiotics. *Best Practice and Research Clinical Gastroenterology*, 18(2): 287-298.

Marshall, R.T., H.D. Goff and R.W. Hartel (2012). *Ice cream*, Springer.

Muse, M.R. and R.W. Hartel (2004). Ice cream structural elements that affect melting rate and hardness. *Journal of Dairy Science*, 87(1): 1-10.

Sanchez, B., C.G. De Los Reyes-Gavilan, A. Margolles and M. Gueimonde (2009). Probiotic fermented milks: Present and future. *International Journal of Dairy Technology*, 62: 472–483.

Santos Leandro, E.D., E. Andrade de Araujo, L. Lopes de Conceicao, L. Alencar de Moraes and A. Fernandes de Carvalho (2013). Survival of *Lactobacillus delbrueckii* UFV H2B20 in ice cream produced with different fat levels and after submission to stress acid and bile salts. *Journal of Functional Foods*, 5 (1): 503-507.

Soccol, C.R., L.P. Vandenberghe, M.R. Spier, A.B.P. Medeiros, C.T. Yamaguishi, J.D.D. Lindner and V. Thomaz-Soccol (2010). The potential of probiotics: a review. *Food Technology and Biotechnology*, 48 (4): 413-434.

Songtummin, S. and B. Leenanon (2016). Survival of *Lactobacillus acidophilus* TISTR1338 and *Lactobacillus casei* TISTR390 in probiotic Gac ice cream. *International Food Research Journal*, 23 (2): 790-796.

Sun-Waterhouse, D., L. Edmonds, S. Wadhwa and R. Wibisono (2013). Producing ice cream using a substantial amount of juice from kiwifruit with green, gold or red flesh. *Food Research International*, 50(2): 647-656.

Tharmaraj, N. and N.P. Shah (2003). Selective Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillusrhamnosus* and Propionibacteria. *Journal of Dairy Science*, 86: 2288–2296.

Turgut, T. and S. Cakmakci (2009). Investigation of the possible use of probiotics in ice cream manufacture. *International Journal of Dairy Technology*, 62(3): 444-451.

(*Accepted for publication November 2018*)