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Evaluation of the Quality Characteristics of Probiotic Ice Cream Produced from a Mixture of Camel Milk and Cow Milk during Frozen Storage

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ABSTRACT

Background: Ice cream is one of the most important and popular frozen desserts based on cow milk that is popular in the world. Due to the valuable properties of camel milk and with the aim of diversifying the production of this product and improving its nutritional quality, **Methods:** In the present study, ratios of 0%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% of camel milk were replaced. The probiotic ice cream formulation contained *Lactobacillus acidophilus* and the samples were stored in a freezer at -18 °C and on the days after (first) production, thirty and sixty days were subjected to physicochemical, microbial, and sensory tests. **Results:** The results showed that the trend of changes in pH, specific gravity and volume increase was irregular. In contrast, the trends of fat changes, melting resistance, and sensory properties were decreasing. In physicochemical tests, treatment based on 40% cow milk had the highest pH value and volume increased, while the highest viscosity and melting resistance were related to camel milk treatment. The highest fat content and specific gravity belonged to 80% camel milk treatment. Treatment based on 40% cow milk showed the highest *L. acidophilus* count, while the treatment based on 60% cow milk had the lowest count. In terms of sensory properties, this treatment received the highest flavor and overall acceptance scores in terms of evaluators, while the highest color and texture score belonged to the treatment based on 40% and 70% camel milk, respectively. **Conclusion:** The overall results showed that cow milk can be replaced by probiotic ice cream formulation with different proportions of camel milk and obtained a desirable product. Finally, treatments based on higher ratios of camel milk had better quality properties and 60% of camel milk was selected as optimum treatment.

Keywords: *Probiotic Ice cream; Camel milk; Lactobacillus acidophilus*

Introduction

Probiotics have been introduced as living effects and optimum concentration (Desmond *et al.*, 2002). The most common probiotic bacteria belong to lactobacillus and bifidobacterium genera. *Lactobacillus casei* is a positive–gram, negative–catalase, mesophyll, microaerophilic, and non-producing spore bacterium (Iyer and Hittinahalli, 2008). Probiotics can provide health benefits on the host upon ingestion in a sufficient number (Shu *et al.*, 2017). Previous research studies

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have proved that probiotics contributed greatly to stronger immunity, lower cholesterol, and blood pressure (Aghajani *et al.*, 2012). The adequate survival of living probiotic should be maintained during shelf-life storage and internal gastro-intestinal tract to benefit human health (De Prisco and Mauriello, 2016).

Among the species of the genus Lactobacillus, *Lactobacillus acidophilus* (*L. acidophilus*) is the most important bacterium used alone or in combination with other bacteria as a fermenting and probiotic bacterium (Eva and Socaciu, 2008). This bacterium needs nutrients to grow and survive (Molin and Mazza, 2008). The researchers concluded that *L. acidophilus* is the most resistant species to gastric juice and bile salts compared to *L. lactis*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. bifidobacteria,* and traditional bacteria (Vinderola and Reinheimer, 2000). Camel milk is white, opaque and has a pleasant but salty taste. The taste of camel milk can change due to the nutrition type, access to water, and the milking condition (Ayadi *et al.*, 2009). The protein range of camel milk is between 2% and 5.5% and is not unlike bovine protein. The average lactose content in camel milk (4.62 %) is slightly lower than cow milk (4.80%). Protein is also a major component of camel milk, which has a significant impact on its nutritional value. Camel milk is similar to human milk in terms of beta-casein content. The high protein content of camel milk increase digestibility and reduce allergenicity in children, which is one of the unique characteristics of camel milk (Shamsia, 2009). Camel milk fat contains small amounts of short-chain fatty acids and carotene, which can be a reason for the whiteness of camel milk (Stahl *et al.*, 2006).

Due to the richness of camel milk and its high nutritional quality, replacing cow milk with this type of milk in different proportions in the probiotic ice cream formulation, in addition to creating variety and producing new products, can improve the quality and increase the storage time of ice cream. The purpose of this study was to evaluate the quality characteristics of probiotic ice cream produced from a mixture of cow milk and camel milk during frozen storage.

Materials and Methods

The raw materials used in the present study included probiotic strain powder of *Lactobacillus acidophilus*-05 (DVS: ATCC 4538; Chr. Hansen, Denmark), MRS bile agar, skim milk powder, phenolphthalein, ringer solution, buffer, methanol (Merck, Germany), distilled water (Tajhiz Azma, Iran), and sorbitol (Merck, Darmstadt, Germany). Cow milk and camel milk were obtained from a supermarket (Alborz, Iran).

Activation of the probiotic strain: Lactobacillus acidophilus was determined on Lactobacillus selective agar plus 0.2% oxgall (LBSO). Plates were incubated at 37°C for 4 days. This strain had previously been shown to demonstrate probiotic properties (Gilliland and Walker, 1990).

Procedure for ice cream manufacturing: Eight ice cream mixes (2 kg each), each of three replicates, were prepared (**Table 1**). All mixes were standardized to contain, 8% fat, 12% milk solids-not-fat, 16% sugar, 0.8% stabilizer/emulsifier, and 0.3% vanilla. In each treatment, mix ingredients were homogenized together as described by (Arbuckle, 2013) and then heated at 80°C for 30 sec. All mixes were cooled to 5°C and aged for 12 h at the same temperature. *L.acidophilus* was cultured for 12 h at 37°C in sterilized skimmed milk, fortified by adding 1% D-glucose (Puriss, Kebbo Lab. AB, 50% w/v filter sterilized solution) and 1% tryptone (Oxoid, 25% w/v filter sterilized solution) as described by (Hagen and Narvhus, 1999). This fermented milk was then added (10% v/v) to eight ice cream mixes prior to freezing. Treatment C was applied as a control sample and prepared by 100% cow milk. Treatments (T1, T2, T3, T4, T5, T6, and T7) were made using mix of cow milk and camel milk based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40:60, respectively (**Table 1**). The freezing was performed in a horizontal batch freezer (Taylor Co., USA). The ice cream was filled in 120 mL plastic cups, covered, and hardened at -26°C for 24 h before analysis.

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Table 1. Specifications of the treatments studied in the present study

Treatments	Cow milk	Camel milk
C	100	0
T1	80	20
T2	70	30
T3	60	40
T4	50	50
T5	20	80
T6	30	70
T7	40	60

The pH measurement: The pH value of the ice cream mixture was measured after the ripening stage using a pH meter (pH meter, Model 691, Metrohm, Swiss) (Aghajani *et al.*, 2012).

Fat measurement: Fat extraction was performed by Soxhlet method. In this method, one gram of the sample was placed inside the sampler of soxhlet and then, several times washed with hexane to extract fat of sample and then the solvent removed by rotary at 41°C (Aghajani *et al.*, 2012).

Overrun measurement: Ice cream overrun was determined on the samples stored at -26°C for 5 days by the following equation (Muse and Hartel, 2004).

Overrun % = 100 × (ice cream volume – mix volume) × (mix volume)$^{-1}$

Analysis of melting resistance: This analysis was performed by weighing the amount of melted ice cream at 25°C for 15 minutes according to (Arbuckle, 2013)) methods.

Mix viscosity analysis: A concentric cylinder viscometer (model LVT, Brookfield, Stoughton, MA) was used to measure the viscosity of 600 ml of ice cream mixture at 4-5°C after 48 h of aging and before freezing. Spindle #2H was used to take torque measurements at 100, 50, 25, 10, 5, 2.5, 1.0, and 0.5 rpm. One measurement was taken per mixture. The shear stress and the shear rate of the mixes were calculated, and the power law model was used to determine the flow behavior index (n) and consistency coefficient (K). The flow behavior index signifies how close the mixtureis to Newtonian. The consistency coefficient gives an indication of the flow properties of the mix. The apparent viscosity was calculated according to the manual for the Brookfield Viscometer (Innocente *et al.*, 2002).

Enumeration of the probiotic strain: Enumeration of *L. acidophilus* was performed on all treatments on the ice cream mixture until the end of the storage time at -18ºC. MRS agar medium (DeMan-Rogosa-Sharpe aga) containing 10 g/l sorbitol was used and the colonies were counted after 72 hr. of incubation at 37 °C and anaerobic condition (Tharmaraj and Shah, 2003).

Sensory test: The sensory analyses were performed with 15 trained panelists using a structured 5-point hedonic scale ranging from 1 (disliked it very much) to 5 (liked it very much). Ice cream was evaluated for color, flavor, texture, and overall acceptance. Approximately 15g of each sample was placed in a 50ml disposable container which was coded with three-digit numbers, sealed and kept in a thermal box to maintain the samples' temperature (approx. 10^{o}C) (Arbuckle, 2013).

Data analysis: All statistical analyses were carried out using the SPSS statistical software program (version 26, SPSS Inc., Chicago, IL USA). Multiple comparisons between means were analyzed with the Duncan's multiple range method at $p<0.05$. All analyses were done in triplicate. Office Excel software was used to draw charts.

Results

Fat content: According to **Figure 1**, the trend of fat changes in the treatments was different over time. There was a significant difference between the control sample and all treatments ($P < 0.05$) (**Figure 1-a**). The highest mean fat content belonged to the treatment based on 80% camel milk (T5) on the thirtieth day, which was significantly different from other treatments and control samples ($P < 0.05$). In general, with increasing the ratio of camel milk in ice cream samples from 20 to 50%, the total fat content decreased (**Figure 1-b**).

Overrun test: According to **Figure 2**, the trend of overrun in most of the treatments was

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incremental. In addition, there was a significant difference between the overrun in the control sample with all the treatments during the first to sixty days ($P < 0.05$) (**Figure 2- b**). The highest overrun belonged to the treatment containing 60% camel milk on the sixtieth day, which was statistically significantly different from other treatments and from the control sample ($P < 0.05$).

Melting resistance: According to **Figure 3-a**, the resistance to melting was reduced in most of the treatments. The highest mean belonged to the control sample on the first day, which showed a significant difference with the treatments ($P < 0.05$). In the present study, control sample and treatment samples based on 20% cow's milk obtained the highest and lowest melting resistance in the whole storage period, respectively, and the difference between the two was significant ($P < 0.05$). There was a significant difference between the control sample and other treatments ($P < 0.05$). The melt resistance of the two treatments based on 60 and 70% camel milk were higher than other treatments and control samples and there was a significant difference between these two treatments together with other treatments ($P < 0.05$, **Figure 3-b**).

Appearance viscosity: According to **Figure 4- a**, the trend of viscosity changes was similar to the trend of changes in melt resistance of treatments during storage. There was a significant difference between all treatments and the control sample ($P < 0.05$). The viscosity values of the treatments containing higher ratios of camel milk were higher. According to **Figure 4-b**, the highest mean viscosity belonged to the treatment based on 70% camel milk, which was significantly different from the control sample and other treatment ($P < 0.05$). There was no significant difference between treatments based on 50% to 80% cow's milk ($P > 0.05$), although the treatment containing 70% cow's milk had the lowest viscosity (**Figure 4-b**).

L. Acidophilus count: According to **Table 2**, the *L. acidophilus* count before freezing and during freezing storage in the treatment based on 70% camel milk was higher than other treatments and control samples. In general, in most of the treatments, during the first to thirty days, there was a significant increase in the *L. acidophilus* count and after that, the bacterial count decreased. Treatments with higher proportions of camel milk showed higher *L. acidophilus* count than cow's milk.

The highest *L. acidophilus* count in storage time of ice cream samples belonged to the treatment based on 70% camel milk, which was significant different from other treatments and control samples ($P < 0.05$). While the lowest count of probiotic strain was related to the treatment based on 40% camel milk, which was significantly different from the control sample ($P < 0.05$). In general, the *L. acidophilus* count was higher in treatments based on higher amounts of camel milk (**Table 2**).

Sensory properties: According to **Figure 5**, the highest color score was related to the treatment based on 60% cow's milk (T3), which was significant with all treatments and control samples ($P < 0.05$). In general, treatments based on higher amounts of camel milk had higher color scores. The treatment based on 60% of camel milk (T7) showed the highest flavor score and its difference with other treatments and control sample was significant ($P < 0.05$). Higher ratios of camel milk resulted in higher flavor scores. The control sample received the highest texture score and its difference was significant with all the treatments ($P < 0.05$). The treatment based on 70% of camel milk obtained the highest texture score that the difference between the mean texture in this treatment with the control sample and other treatments is significant ($P < 0.05$). However, the highest overall acceptance score belonged to the treatment based on 60% of camel milk, which was significantly different from the control sample and other treatments ($P < 0.05$) (**Figure 5**).

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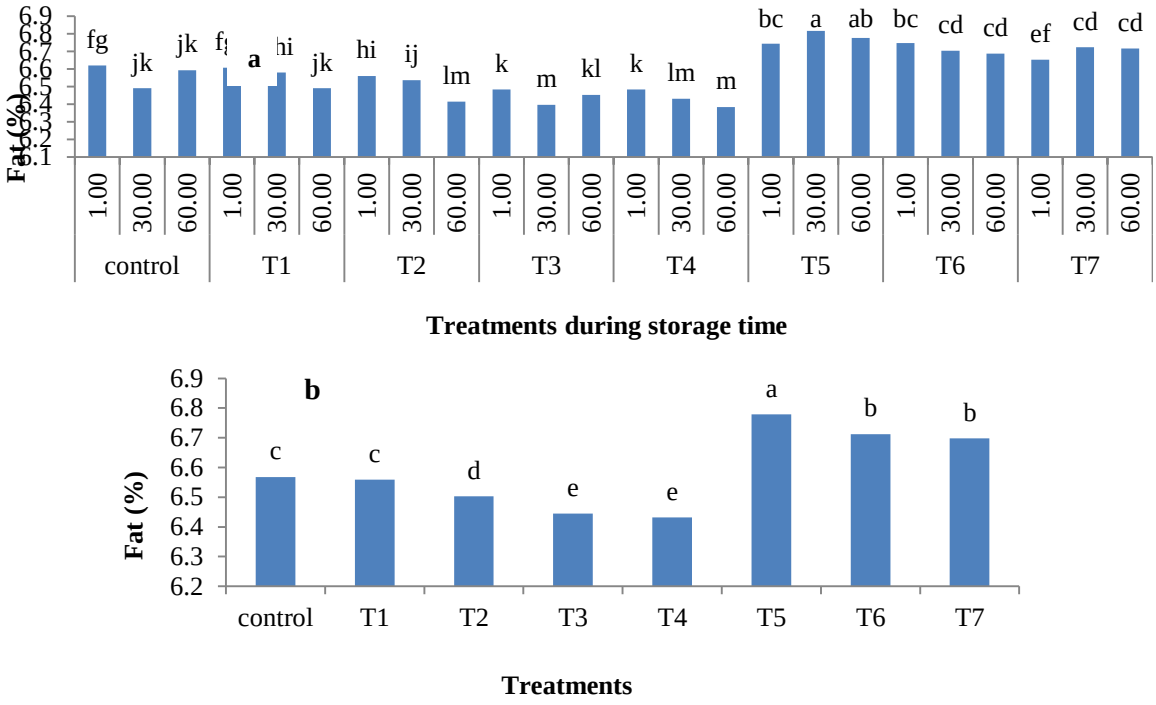

Figure 1. The trend of changes (a) and comparison of the mean (b) of fat content in ice cream samples during frozen storage. The letters T1, T2, T3, T4, T5, T6, and T7 represent treatments based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40: 60 cow milk: camel milk and the letter C represents the sample based on 100% cow milk.

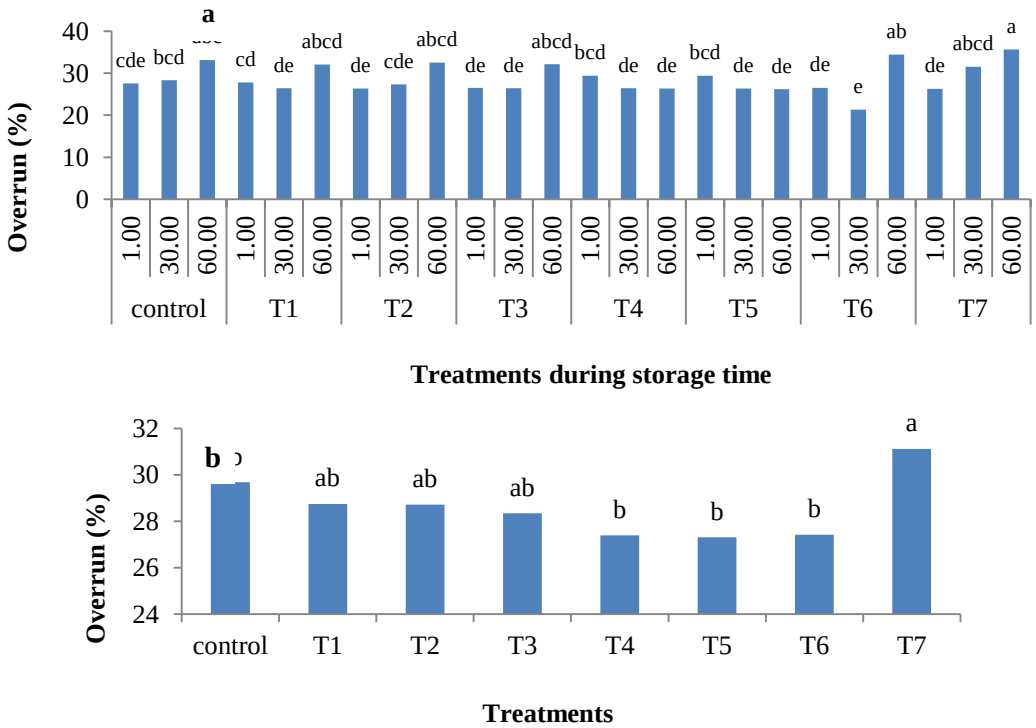

Figure 2. The trend of changes (a) and comparison of the total mean overrun (b) of ice cream samples during frozen storage. The letters T1, T2, T3, T4, T5, T6, and T7 represent treatments based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40:60 cow milk: camel milk and the letter C represents the sample based on 100% cow milk.

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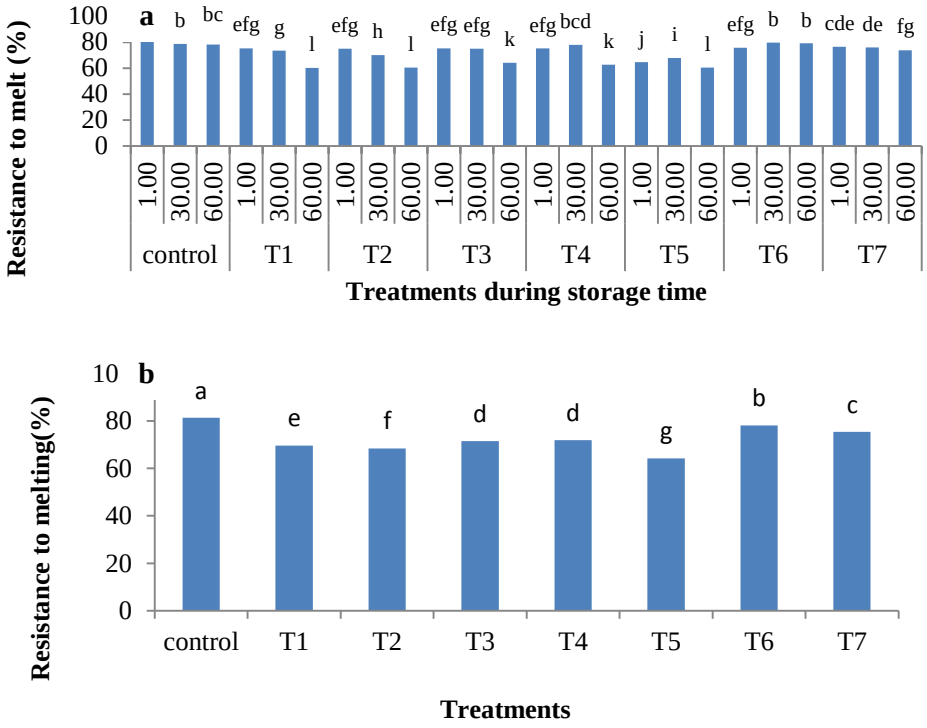

Figure 3. The trend of changes (a) and comparison of the total mean (b) melting resistance of ice cream samples during frozen storage. The letters T1, T2, T3, T4, T5, T6, and T7 represent treatments based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40:60 cow milk: camel milk and the letter C represent the sample based on 100% cow milk.

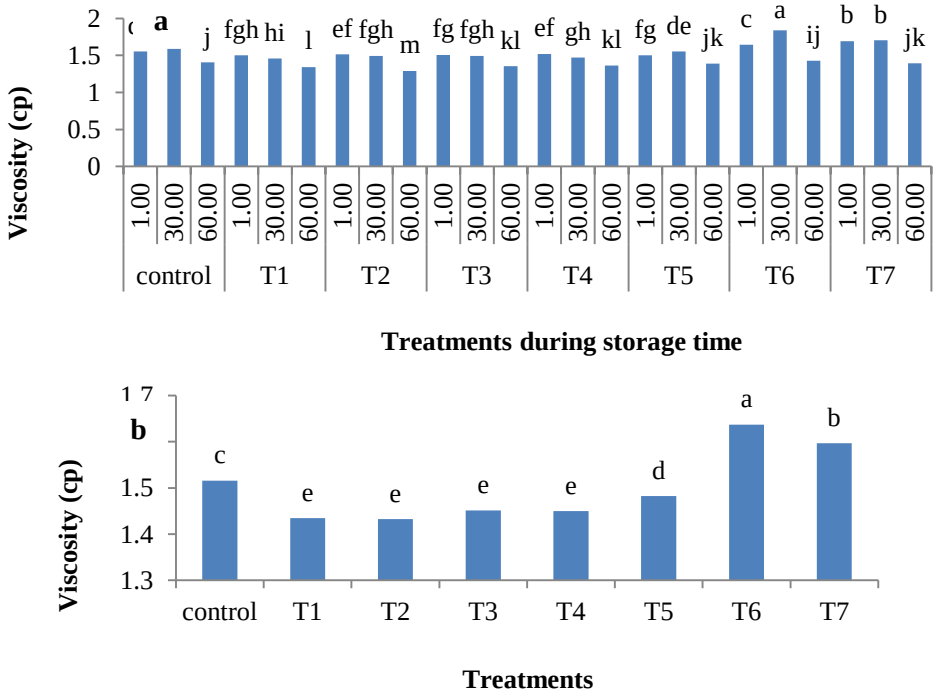

Figure 4. Changes trend (high) and comparison of the total mean (low) of melting resistance of ice cream samples during frozen storage. The letters T1, T2, T3, T4, T5, T6, and T7 represent treatments based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40:60 cow milk: camel milk and the letter C represents the sample based on 100% cow milk.

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Table 2. The trend of changes in the *L. acidophilus* count (log cfu/ml) in ice cream samples during frozen storage.

Time	Treatments							
	C	T1	T2	T3	T4	T5	T6	T7
Before freezing	9.00±0.02^{a}	8.21±0.01^{c}	8.49±0.01^{b}	8.19±0.01^{c}	8.49±0.01^{b}	8.30±0.01^{c}	9.1±0.01^{a}	9±0.01^{a}
First day	8.29±0.11^{c}	8.11±0.01^{d}	8.36±0.01^{c}	8.03±0.01^{d}	8.38±0.01^{c}	8.51±0.01^{b}	8.70±0.01^{a}	8.67±0.01^{a}
Thirtieth day	8.33±0.01^{b}	8.34±0.01^{b}	8.46±0.01^{a}	8.29±0.01^{b}	8.42±0.01^{a}	8.48±0.01^{a}	8.51±0.01^{a}	8.50±0.01^{a}
Sixteenth day	7.30±0.01^{b}	7.13±0.01^{b}	8.0±0.01^{a}	7.13±0.01^{b}	7.68±0.01^{b}	8.0±0.01^{a}	8.10±0.01^{a}	8.06±0.01^{a}

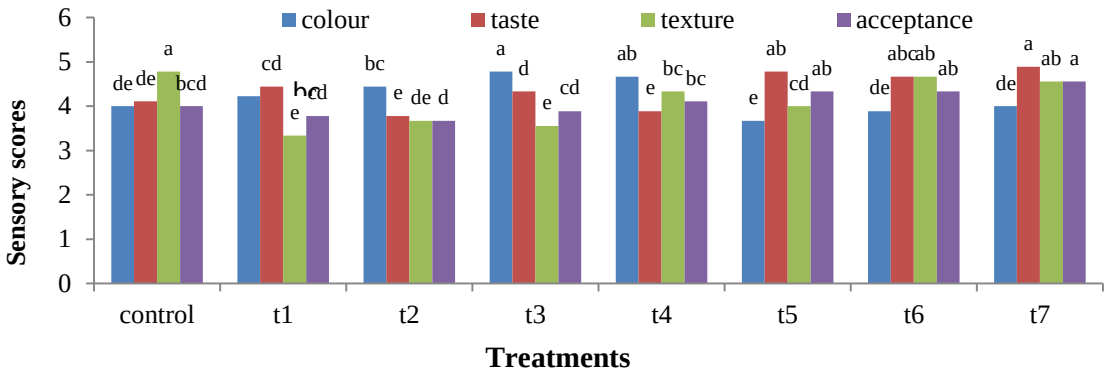

Figure 5. Comparison of the mean of all sensory parameters of treatments without considering time. The letters T1, T2, T3, T4, T5, T6, and T7 represent treatments based on 80:20; 70:30; 60:40; 50:50; 20:80; 30:70, and 40:60 cow milk: camel milk and the letter C represents the sample based on 100% cow milk (control).

Discussion

Camel milk fat contains a small amount of short-chain fatty acids and carotene, which this pigment can be the reason for the whiteness of camel milk (Stahl *et al.*, 2006). This milk has higher amounts of unsaturated fatty acids than cow's milk (Niasari-Naslaji *et al.*, 2012).

The successful processing of ice cream from camel milk might indicate the possibility of using camel milk to produce special ice cream such as low fat ice cream (Ahmed and El Zubeir, 2015). This supported (Abu-Lehia *et al.*, 1989), who reported that the overrun of camel milk ice cream was found to significantly depend on the fat and Milk Solids Not Fat (MSNF) levels in the mixture.

The overrun compared to the mixture volume is due to the entry of air into it through a whipping and aeration, and this overrun is influenced by various parameters such as the ingredients type such as fat, total solid, stabilizers and sweeteners and the freezing equipment type. The amount of air that enters the ice cream is important for two reasons: its relationship with efficiency and profitability and its effect on the texture, body and overall acceptance of ice cream (López-Rubira *et al.*, 2005). Larger ice crystals have been observed in ice cream with a low -volume increase. Often, the ice creams with low overrun had firmer textures (Moeenfard and Tehrani, 2008). According to **Figure 2-a**, a decreasing trend was observed in some treatments, in which case, Ghanbari and Farmani (Ghanbari and Farmani, 2013) attributed the decrease in overrun to the drop in freezing point .In some studies, a decrease in ice cream volume was reported as a result of a significant increase in viscosity (Bahramparvar *et al.*, 2009).

The ice cream melting depends on several factors such as the amount of air entering the ice cream, the shape and growth of ice crystals, the network of fat globules formed during freezing and ice cream firmness (Rezaei *et al.*, 2011). As the ice cream viscosity increases, the resistance to melting also increase (Mohammadi Sani, 2015). The higher viscosity of ice cream reduces the mobility of water molecules and their movement between the

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mixture molecules, thus improving the resistance to melting (Jooyandeh *et al.*, 2017). Therefore, a logical relationship can be established between viscosity and melt resistance. Milani and Koocheki reported that increasing the consistency and viscosity of ice cream mix can improve the melt resistance of ice cream samples (Milani and Koocheki, 2011). Javidi *et al.* reported that increasing the viscosity, increased the melt resistance of ice cream samples (Javidi *et al.*, 2015). Instability of milk fat has the greatest effect on the melting rate (Muse and Hartel, 2004). Researchers attribute fat instability to the high viscosity and type of ice cream ingredients. It has also been reported that increasing the viscosity of ice cream increases its resistance to melting (Herald *et al.*, 2008). Decreasing the melting of the ice cream can be attributed to the increase in viscosity and stability of the ice cream emulsion. Therefore, it can be said that all mechanisms effective in increasing the viscosity and emulsion stability affect the melting rate of the ice cream (Guarda *et al.*, 2004). The viscosity values of the treatments containing higher ratios of camel milk were higher. The main reason for the increase in viscosity seems to be due to the presence of more protein in camel milk compared to cow's milk in ice cream. Therefore, the presence of these high molecular weight compounds justifies the increase in viscosity by binding to water and forming a gel network. The viscosity changes in this study were similar to the findings of Ozkoc (Ozkoc *et al.*, 2009). Rosell *et al.* found that the reason for the increase in viscosity of low-fat ice cream contained in sago fruit was the lack of starch and high protein in this fruit and stated that increasing gelatinization of starch may increase the water holding capacity (WHC) and viscosity of the fruit (Rosell *et al.*, 2001). The higher the viscosity of the liquid, the greater the shear stress required for the same deformation. The milk composition and its dry matter along with parameters such as temperature, heating time and starter type used and storage conditions are effective factors in the rheological properties of the final product (Girard and Schaffer-Lequart, 2007). It can be said that increasing the viscosity of ice cream mix leads to a decrease in ice crystallization and thus a decrease in ice cream firmness (Romanchik-Cerpovicz *et al.*, 2002). Increasing the viscosity of the mixture leads to a reduction in ice crystallization and thus a decrease in the ice cream hardness (Romanchik-Cerpovicz *et al.*, 2002). Increased viscosity with increasing storage time can be due to protein rearrangements and protein-protein binding changes (Sahan *et al.*, 2008).

One of the reasons for the increase in viscosity can be attributed to the production of exopolysaccharides (EPSs) by *Lactobacillus acidophilus*, which will increase with increasing ice cream storage time (Bahramparvar *et al.*, 2009). This increase can be due to more covalent and hydrogen bonds, more water absorption and stronger texture during storage in the ice cream structure (Duboc and Mollet, 2001). In the case of changes in fat content of camel milk, it was observed that with increasing fat content, viscosity also increased significantly and this result shows the texturizing role of fat in dairy products, so, by increasing the fat content of primary camel milk, the product will have a higher viscosity and consistency (Vasiljevic *et al.*, 2007).

The findings of this study are consistent with the results of other researchers who reported a significant decrease in the count of *L. acidophilus* during the freezing process (Akalın and Erişir, 2008, Magarinos *et al.*, 2007, Nousia *et al.*, 2011). *L.acidophilus* produces extracellular and intracellular enzymes that can hydrolyze biological active peptides and bradykinin (Donkor *et al.*, 2007). Therefore, *L.acidophilus* consumes the ice cream protein and by converting it into new peptides, especially bioactive substances, increases the nutrients available for growth and leads to increased *L.acidophilus* growth (Gonzalez-Gonzalez *et al.*, 2011). Although, some other researchers have reduced the *L.acidophilus* count was reported during the freezing process, but this decrease was not significant compared to the Lactobacillus population in the ice cream mix (Turgut and Cakmakci, 2009). Hekmat and McMahon reported that the survival of

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L.acidophilus in ice cream mix decreases by 2 logarithmic cycles after 17 weeks of storage at -29°C (Hekmat and McMAHON, 1992). Decrease in the *L.acidophilus* is consistent with the findings of Akin et al. and Hekmat and McMahon during storage at freezing temperatures, but contradicts the findings of other researchers (Akalın and Erişir, 2008, Turgut and Cakmakci, 2009).

The flavor of camel milk can change due to the nutrition type, access to water and the number of milking's (Ayadi *et al.*, 2009). The resistance of ice cream to the mechanical forces created by the tongue, palate, and teeth determines the overall understanding of the ice cream texture (Aime *et al.*, 2001). The flavor characteristics of ice cream are one of the most important factors in its overall acceptance (Soukoulis *et al.*, 2008). Flores and Goff reported that milk proteins have a significant effect on ice cream texture by limiting the size of ice crystals, and this property is intensified by polysaccharides (Flores and Goff, 1999). One of the reasons for the decrease in firmness can be attributed to the increase in overrun. Many researchers have found that increasing the overrun, reduces the hardness (Romanchik-Cerpovicz *et al.*, 2002). Another reason can be stated that with the emergence of large ice crystals, the hardness increases, which is achieved through the control of crystallization and water (Mariotti *et al.*, 2006). The reduction in hardness may be attributed to the freezing concentration in the serum phase. As shown in **Figure 5**, higher ratios of camel milk in the ice cream formulation resulted in higher overall acceptance scores.

Conclusion

The results of the present study showed that by increasing storage time, the increase in overrun had irregular changes; however, the trend of fat changes, melting resistance, and sensory properties were decreasing. Treatment based on 40% cow milk had the highest overrun and treatment based on camel milk had the highest viscosity and melting resistance. The highest fat content belonged to the treatment based on 80% camel milk. There was a direct relationship between viscosity values and melting resistance in probiotic ice creams. Treatment based on 70% camel milk showed the highest count of *L. acidophilus*, which showed the effect of compounds in camel milk in ice cream formulation on growth stimulation and activity of this probiotic strain. However, the count of this strain at the end of the 60th day is also within the standard range, so these treatments can be introduced as probiotic. Treatment based on 40% cow milk obtained the highest flavor and overall acceptance scores, while the highest color and texture scores belonged to the treatment based on 40% and 70% camel milk, respectively. Finally, treatments based on higher proportions of camel milk had better quality properties and treatment based on 60% camel milk was selected as the optimal treatment.

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Authors' contribution

Mazreati M: Data curation, Formal analysis, Investigation, Methodology, Writing and original draft. Nateghi L: Funding acquisition, Project administration, Supervision, Writing, review & editing.

Conflict of interest

The authors report no conflicts of interest.

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