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# Probiotic Frozen Yoghurt Production Using Camel Milk (*Camelus dromedarius*) with Improved Functions by Strawberry Guava (*Psidium littorale var. cattleianum*) Fortification

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author NK designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author GK managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.*

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## ABSTRACT

In this study, Probiotic Yoghurt (PY) was produced by adding skimmed milk powder (5% w/v) and probiotic culture (7% w/v) (*Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium* ssp.) to heat-treated camels (*Camelus dromedarius*) milk. Sahlep (*Orchis sanctum* L.) (2% w/v) and skimmed milk powder (15% w/v) were added to the camel milk which was then divided into two batches. The 1st batch was fortified with 20% strawberry guava (*Psidium littorale var. cattleianum*). The 2nd batch was prepared without guava fruit fortification. The mixture was then homogenized with probiotic yoghurt at a 40:60 ratio to yield probiotic guava frozen yoghurt (GPFY) and plain Probiotic Frozen Yoghurt (PFY) samples. Samples were stored for 120 days at -20±1℃. Physicochemical, microbiologi cal and sensory analyses were conducted on the 1st, 15th, 30th, 60th and 120th days of storage. A significant relationship was found between

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the sahlep and fruit fortification with the physicochemical properties of the ice creams ($P< .05$). Vitamin C losses were low in GPFY samples. During storage, *Lactobacillus bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium* ssp. counts in PFY samples first decreased and then increased. The decrease in GPFY was lower and the increase higher than in PFY. *Streptococcus thermophilus* decreased in PFY during storage whereas in GPFY it decreased until the 30th day and increased in the further days of the storage. In all PFY samples, the relationships between fat content, sahlep ratio and fruit fortification with the viability of the microorganisms were significant ($P< .05$).

*Keywords: Lactobacillus acidophilus; Bifidobacterium ssp; Sahlep; frozen yoghurt; camel milk; strawberry guava (Psidium littorale var. cattleianum).*

## 1. INTRODUCTION

Frozen yoghurt is a functional food produced from the mixture of ice cream and yoghurt. It contains high levels of live yogurt culture and has the combined characteristics of ice cream and yoghurt. It is produced by two different methods. In the first method, an ice cream mixture is inoculated with starter cultures, fermented and frozen. In the second method, first the ice cream mixture is prepared, then a certain amount of yoghurt is added [1]. Frozen yoghurt is produced by adding classic yoghurt culture, various flavorings, stabilizers [4], fruits, cereals, and plants [2-4], prebiotics [5], and microbial-derived enzymes [6] to different milks [cow, goat, ewe]. Frozen yoghurts, as well as classic starter cultures, are produced with probiotic cultures such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus* [7].

Probiotic microorganisms may be therapeutic effects on health when taken in sufficient amounts [8]. These effects vary depending on the probiotic species or strains, and also on the food matrix. Therapeutic effects appear when the microorganism counts in the final product are at $10^7$-$10^8$cfu$^{-g}$ [8]. Ranadheera et al. [9] produced probiotic ice creams using goat's milk and stored them at -20℃ for 24 weeks. They reported that additives including cocoa powder and stabilizers (guar gum and dextrose), due to their high fat contents, preserved the viability of the probiotics. In the studies on the relationship between probiotics and prebiotics, it has been reported that resistant starches were more effective on the development of *Bifidobacterium ssp*., compared to inulin and raftilose (oligofructose) [10].

In Turkey, the hydrocolloid stabilizer sahlep (*Orchis sancta L.*) is used in the production of Maraş and Maraş type ice cream. Sahlep, in addition to its stabilizing function, adds taste and aroma to the product. Depending on the type, it contains 11.6% - 55.4% glucomannan in its composition. It provides a smooth, homogeneous texture to the ice cream, delays melting and increases viscosity as its concentration increases. Overrun seen in ice cream with sahlep fortification was reported to be higher than those of ice creams containing other stabilizers (carob, carboxymethyl cellulose, gelatin, gum arabic and soapwort root) [11,12].

There are a number of studies on production of yoghurt, probiotic yoghurt [13], stabilizer-supplemented yoghurt [14], yoghurt supplemented with different spices [15] and flavored yoghurt [16] from camel milk. Quantitative studies on changes in industrial and probiotic yoghurt cultures have also been conducted [17,18].

Guava is rich in vitamins A and C, folic acid, dietary fiber, carotenoids, phenolic compounds, and minerals such as calcium, potassium and iron [19,20]. The level of vitamin C (228 mg/100 g) is four times that of orange [21]. It is an important source of antioxidants due to its vitamin C and beta-carotene contents [22]. Additionally, it contains lycopene, lutein and zeaxanthin [21,23]. Guava is rich in terms of tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Flavonoids exhibit antimicrobial and antidiabetic properties [24].

In this study, probiotic yoghurt (PY) was produced by adding 7% probiotic yoghurt culture *Streptococcus thermophilus* (*Str.thermophilus*) + *Lactobacillus bulgaricus* (*Lb. bulgaricus*) +*Lactobacillus acidophilus* (*Lb.acidophilus*) and *Bifidobacterium ssp*. to raw camel (*Camelus dromedarius*) milk (CaM) which was standardized to 14% dry matter content with skimmed milk powder fortification (5% w/v) at a 1:1 ratio. Ice cream mixture was prepared with 15% skimmed milk powder (w/v), 2% sahlep (w/v), 14% sugar (w/v) and 20% strawberry guava pulp (w/v). As a control, guava pulp was not added to one batch of the ice cream mixture. Two different types of probiotic frozen yoghurts were produced (GPFY and PFY) by direct yoghurt addition to the ice cream. Samples were stored for 120 days at -20±1℃. Physicochemical, microbiological and sensory analyses were conducted on the 1st, 15th, 30th, 60th and 120th days of storage. Trials were carried out in triplicate.

## 2. MATERIALS AND METHODS

Raw camel milk (*Camelus dramedarius*) (CaM) was obtained from a local camel farm located in Denizli Sarayköy, Turkey. Sahlep (*Orchis sancta L*.), produced from tubers collected from different parts of Turkey (composition: 32.25% dry matter, 3.50% fat , 3.15% protein, 2.9% starch, 11% moisture, 2.1% minerals. pH 6.49) was obtained from a local business (Turkey), strawberry guava (*Psidium littorale var. cattleianum*) was obtained from a local producer in Mersin, Turkey, skimmed milk powder was obtained from Pınar Milk Products Inc. (Turkey), and sugar used in the ice cream mixture was obtained from Kent Inc. (Turkey). Probiotic yoghurt culture YO-MIX 205 (*Str. thermophilus + Lb. bulgaricus, +Lb. acidophilus*) (Danisco-FRANCE) and BIFI freeze-dried yogurt culture (*Bifidobacterium ssp*.) was obtained from CSL laboratories (Strade per Merlino, 3- 26839, Italy). Yoghurt, ice cream mix and probiotic frozen yoghurt were produced in Pilot Plants in Ege University, Faculty of Agriculture, Department of Dairy Technology.

### 2.1 Probiotic Yoghurt (PY) Production

Raw camel milk which was standardized to 14% dry matter content with skimmed milk powder fortification (5% w/v) was pasteurized at 85℃ for 15 minutes. Then, it was cooled to 45±1℃ and inoculated with probiotic starter culture mixture (1:1) (*Str. thermophilus + Lb. bulgaricus,+ Lb. acidophilus* and *Bifidobacterium ssp*.) at 7%. Samples were placed into plastic cups (200 g) and incubated at 43±1℃ (approximately 11 hours), after which pH was 4.8-4.9. Physicochemical analyses were performed on the samples. The ratio of starter culture was determined as a result of preliminary trials. The ratios of cultures tested in the preliminary trials were 3% (v/v), 5% (v/v) and 7% (v/v). It was determined that a more viscous and stiff structure was obtained with the 7% starter culture. Skimmed milk powder ratio was determined bya similar approach. These results were compatible with those reported by Hashim et al. [16].

### 2.2 Ice Cream Mix Production

CaM was heated to 30℃ and 15% milk powder (w/v) and 2% salep (w/v) were added. Temperature was increased to 55℃ and 14% sugar was added. The mixture was then pasteurized at 90℃ for 10 minutes. Then the mixture was divided into two batches and cooled to 37℃. The first batch was prepared without guava fortification while the 2nd batch was fortified with pasteurized guava pulp (20%). During the preparation of the mixtures, the mixtures were homogenized using Ultra Turrax Blender at 1200 rpm for 40s (IKA, Merc, Germany). Finally the mixtures were left to mature at +4±1℃ for 24 hours. Sahlep (2% w/v) and guava pulp (20% w/v) ratios were determined as a result of the preliminary trials. Sahlep ratios tested in preliminary trials were 1% (w/v), 1.5% (w/v) and 2% (w/v). Viscosity, melting resistance and firmness values were low with 1% (w/v) and 1.5% (w/v) sahlep ratios, whereas these values were high with 2% (w/v). Guava pulp ratios added to the mixtures in preliminary trials were 10% (w/v), 20% (w/v) and 30% (w/v). In the preliminary trials, it was determined that 10% (w/v) guava was not perceived in the mixture whereas 30% (w/v) guava caused some textural problems such as rough texture and appearance.

### 2.3 Preparation of Strawberry Guava Pulp

Strawberry guava fruit was provided fresh, centrifuged using an Ultra Turrax Blender (1200 rpm, 40s) (IKA, Merc, Germany) and removed from its seeds by filtering through a wire strainer. Guava pulp was pasteurized at 80℃ for 60s [25].

### 2.4 Probiotic Yoghurt (PY) Ice Cream (IC) Production

The production of PFY (containing no guava in the mixture) and GPFY containing 20% w/v guava are shown in Fig. 1. In this study, yoghurt was added directly into the mixture. Accordingly, probiotic yoghurt was produced from camel milk and mixture was mixed at 40:60 ratio (v:v). The mixture was homogenized in a batch-freezer (Sevel brand, L/40-4 model-Turkey) for approximately 15 minutes until the mixture became homogeneous. PYI samples were placed into plastic cups (80 g) and stored at -20±1℃ for 120 days. Frozen yoghurt production was carried out in triplicate.

## 2.5 Analyses of CaM, PY, Ice Cream Mixture and PFY Analyses

### 2.5.1 Physical-chemical analysis

In CaM and PY samples, dry matter (Binder ED-53, Germany), ash (Protherm PFL 110/6, Turkey) were determined using gravimetric methods. Fat measurement (%) was conducted using the Gerber method while titratable acidity was determined as percent lactic acid. pH was determined using an SS-3 Zeromatic pH meter (Beckman Instruments Inc., California, USA). Protein was determined by the Kjeldahl method [26]. A texture analyzer (Brookfield CT3 4500 model, USA/ Shape Cylinder; Target 10 mm; Test speed 1 mm/s) was used for texture analysis and viscosity was determined as cP [27] using a Brookfield Digital Viscometer (Model DV-II+PRO, USA) [180 rpm, at 10℃, LV2 spindle (23.47 g), between 13-42% Torque]. Overrun analysis in PFY samples were conducted according to Metin [28]. Overrun was calculated as % (OR %) using the equation below. Melting ratios in PFY samples were determined according to Gürsel and Karacabey [29].

OR%= ((MM-IM) x 100/IM)

OR: Overrun (%)

IM: Ice -cream Mass (g)

MM: Mass of the melted ice cream (g)

### 2.5.2 Vitamin C analysis

Vitamin C contents were measuredin guava fruit and in PFY samples during storage. Accordingly, 25g samples were grinded in a Waring commercial blender (Blender 8011ES, ABD) with the addition of 100 mL oxalic acid (0.4%) and filtered through paper filter. Then, 9 mL oxalic acid was added to the 1 mL sample taken from the filtrate. Vitamin C contents (L-ascorbic acid) were determined by titrating with 2,6-dichloroindophenol according to AOAC titrimetric method [30] and measured in a spectrophotometer (Varian Bio 100, Australia) at 518 nm. The results were given as mg vitamin C/100 g.

### 2.5.3 Microbiological analyses

Enumerations of starter cultures in yoghurt samples were conducted according to the International Dairy Federation standard method [31,32]. *L. acidophilus* and *Bifidobacterium* ssp. counts were determined according to International Dairy Federation standard methods [33,34].

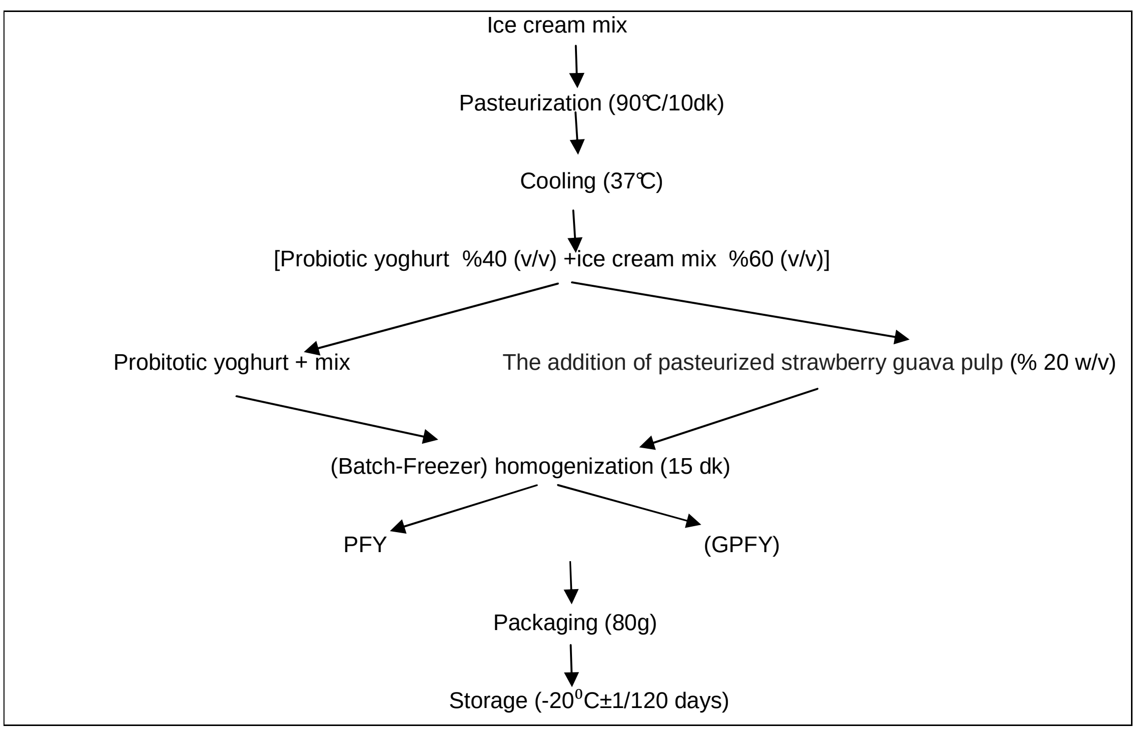


**Fig. 1. Probiotic frozen yoghurt production**

#### 2.5.4 Sensory analyses

The sensory evaluation of yoghurts was carried out with a consumer acceptance test [35] based on the appearance, texture, flavor, aroma, and overall impression of the product using a 9-point hedonic scale (1-disliked extremely; 9-liked extremely). Sensory evaluation of the yoghurt samples were performed after 1 and 10 days of refrigerated storage.

#### 2.5.5 Statistical analysis

Samples were examined with 3 parallels and 2 repetitions using SPPS version 15 (IBM SPSS Statistics) statistical analysis software. Data found significant as a result of analysis of variance (ANOVA) were tested according to the Duncan multiple comparison test at $P < .05$ level.

## 3. RESULTS AND DISCUSSION

### 3.1 Physicochemical Properties of Camel Milk (CaM), Probiotic Yoghurt (PY) and Mixture

Physicochemical Properties of Camel Milk (CaM), Probiotic Yoghurt (PY) and Mixture are shown in Table 1.

Dry matter of strawberry guava was 20.57% and the vitamin C level was 173.12 mg/100 g. Dry matter, viscosity, titratable acidity and ash values increased in PY samples and in the ice cream mixture. In PY samples, the decrease in protein values was significant whereas the decrease in protein in the mixture was not significant. Fat values did not change in PY samples and decreased in the mixture. In PY production, the relationships between milk powder (5% w/v) and starter culture level (7% v/v) to the increases in dry matter, viscosity, titratable acidity and the decrease in protein was significant ($P< .05$). The change in fat was not significant ($P > .05$). Our study results were compatible with previous studies reporting the occurrence of some problems during fermentation in production using camel milk including a lack of change inviscosity during gelation [18]. However, the study was compatible with previous studies reporting long incubation periods (approximately 11 hours), the possibility of yoghurt production using high ratios of milk powder and yoghurt [29] and a better development when using combined yoghurt cultures [16].

In the plain yoghurt and the sample prepared with sahlep and guava, dry matter and viscosity increased while acidity, fat and protein values decreased although the changes were more pronounced with guava pulp addition. The relationship between the increase in dry matter and viscosity due to guava pulp fortification, and also the decrease in acidity and fat were significant ($P< .05$), while the relationship between pulp fortification and the decrease in protein was not significant ($P > .05$). Dry matter and viscosity values in the mixture containing pulp were higher compared to those in the plain mixture whereas the increase in titratable acidity was higher. In a general evaluation, the increase in dry matter and viscosity was associated with stabilizer (sahlep) and fruit ratio. This result was compatible with previous studies reporting that stabilizers improve the functional properties of ice cream [36,37].

### 3.2 Physicochemical Results for Probiotic Frozen Yoghurt

Physicochemical changes during storage of GPFY produced with 20% (w/v) pulp fortification and PFY produced as plain probiotic frozen yoghurt and vitamin C levels in GPFY are shown in Table 2. Dry matter increased in GPFY and PFY between the 1st and the 120th days of storage. The increase in GPFY was higher than in PFY. The relation between fruit pulp addition and the increase in dry matter was significant ($P< .05$). Overrun and melting resistance increased during storage. Overrun and melting resistance of GPFY was higher than of PFY. The relationships between guava pulp and sahlep fortification with overrun and melting resistance were significant ($P< .05$). It has been reported that stabilizer addition, due to the increase in viscosity, leads to the formation of air bubbles which increases overrun, water holding and micro-viscosity and thus melting resistance [38]. Güven and Karaca [39] determined that an increase in sugar and fruit content caused an increase in overrun and viscosity. The study results were not compatible with previous studies reporting that viscosity, melting resistance and hardness in ice creams produced with only sahlep fortification were lower than those produced with fortification with other stabilizers [2]. On the other hand, the results were compatible with studies reporting that sahlep fortification increased the melting resistance of ice cream and provided an overrun [12]. The sahlep ratio used in our study (2%) was higher than those reported in the literature (0.2 - 0.5% w/v). Viscosity, melting resistance and stiffness hardness levels increased as the sahlep concentration increased.

**Table 1. Physicochemical properties of Camel Milk (CaM), Probiotic Yoghurt (PY) and mixture**

| | Camel milk | Probiotic Yoghurt (PY) | Ice cream mixture |
|---|---|---|---|
| Drymatter (%) | 10.88 | 16.5 | 50.85 |
| Viscosity (cP) | 1.28 | 118.5 | 119.46 |
| pH | 6.18 | 4.66 | 5.43 |
| Titratable acidity (LA%) | 0.131 | 1.190 | 0.560 |
| Fat (%) | 2.81 | 2.72 | 4.11 |
| Protein (%) | 2.77 | 3.54 | 5.76 |
| Ash (%) | 1.48 | 2.39 | 5.17 |

**Table 2. Physicochemical properties of GPFY and PFY samples and vitamin C content of GPFY (n=3)**

| | Time storage | GPFY | PFY |
|---|---|---|---|
| **Total solid (%)** | 1st day | 23.47±2.23$^{aA}$ | 21.47±2.34$^{aB}$ |
| | 15 th day | 25.31±2.05 $^{Aa}$ | 22.85±2.13$^{aB}$ |
| | 30 th day | 27.48±2.32$^{aA}$ | 23.47±2.22$^{aB}$ |
| | 60 th day | 29.20±2.34$^{aA}$ | 25.05±2.19$^{aB}$ |
| | 90 th day | 33.61±3.01$^{bA}$ | 26.21±2.18$^{bB}$ |
| | 120 th day | 34.18±3.15$^{bA}$ | 28.84±2.44$^{bB}$ |
| **Viscosity (cP)** | 1st day | 3273.11±23.25$^{aA}$ | 2425.26±20.44$^{aB}$ |
| | 15 th day | 3820.10±24.13$^{bA}$ | 2541±22.43$^{aB}$ |
| | 30 th day | 4649±29.52$^{cA}$ | 2763.12±25.44$^{aB}$ |
| | 60 th day | 5220±30.25$^{dA}$ | 3125.10±28.34$^{bB}$ |
| | 90 th day | 5348±30.13$^{dA}$ | 3354.56±28.56$^{cB}$ |
| | 120 th day | 5847±31.14$^{eA}$ | 3441.78±28.69$^{cB}$ |
| **Hardness (1/10mm)** | 1st day | 105.57±10.25$^{aA}$ | 78.14±1.34$^{aB}$ |
| | 15 th day | 149.05±10.27$^{aA}$ | 94.57±9.34$^{bB}$ |
| | 30 th day | 297.21±10.40$^{bA}$ | 121.26±10.33$^{cB}$ |
| | 60 th day | 324.15±13.14$^{cA}$ | 175.49±10.27 $^{dB}$ |
| | 90 th day | 328.11±13.28$^{cA}$ | 194.45±10.46$^{eB}$ |
| | 120 th day | 331.24±14.05$^{dA}$ | 211.11±10.23$^{fB}$ |
| **Titration Acidity (LA %)** | 1st day | 0.489±0.25$^{aA}$ | 0.376±0.04$^{aB}$ |
| | 15 th day | 0.629±0.13$^{aA}$ | 0.429±0.14$^{aB}$ |
| | 30 th day | 0.691±0.10$^{aA}$ | 0.596±0.11$^{aB}$ |
| | 60 th day | 0.697±0.17$^{aA}$ | 0.657±0.22$^{aB}$ |
| | 90 th day | 0.719±0.13$^{aA}$ | 0.687±0.21$^{aB}$ |
| | 120 th day | 0.721±0.19$^{aA}$ | 0.701±0.24$^{aB}$ |
| **Fat (%)** | 1st day | 6.2±1.03$^{aA}$ | 6.81±1.05$^{aB}$ |
| | 15 th day | 6.19±1.04$^{aA}$ | 6.80±1.02$^{aB}$ |
| | 30 th day | 6.18±1.03$^{aA}$ | 6.80±1.02$^{aB}$ |
| | 60 th day | 6.18±1.02$^{aA}$ | 6.79±1.03$^{aB}$ |
| | 90 th day | 6.17±1.00$^{aA}$ | 6.79±1.05$^{aB}$ |
| | 120 th day | 6.16±1.01$^{aA}$ | 6.79±1.07$^{aB}$ |
| **Protein (%)** | 1st day | 5.67±0.97$^{aA}$ | 5.71±1.00$^{aA}$ |
| | 15 th day | 5.47±0.93$^{aA}$ | 5.68±1.00$^{aA}$ |
| | 30 th day | 5.17±0.88$^{aAaA}$ | 5.52±0.99$^{aA}$ |
| | 60 th day | 4.90±0.90$^{aA}$ | 5.09±0.97$^{aA}$ |
| | 90 th day | 4.64±0.92$^{aA}$ | 4.78±0.93$^{aA}$ |
| | 120 th day | 4.31±0.80$^{aA}$ | 4.54±0.87$^{aA}$ |
| **Overrun (%)** | 1st day | 20.44±5.40$^{aA}$ | 17.25±4.34$^{aB}$ |
| | 15 th day | 24.44±4.25$^{aA}$ | 19.65±4.58$^{aB}$ |
| | 30 th day | 27.10±5.30$^{aA}$ | 22.45±4.67$^{aB}$ |
| | 60 th day | 39.73±4.40$^{bA}$ | 29.78±4.24$^{aB}$ |

| | Time storage | GPFY | PFY |
|---|---|---|---|
| | 90 th day | 43.75±4.33$^{cA}$ | 35.41±4.90$^{bB}$ |
| | 120 th day | 76.69±6.25$^{dA}$ | 44.14±5.14$^{cB}$ |
| **Melting (%)** | 1st day | 26.90±3.45$^{aA}$ | 24.16±4.04$^{aB}$ |
| | 15 th day | 40.57±3.50$^{bA}$ | 34.48±4.32$^{bB}$ |
| | 30 th day | 40.98±3.68$^{bA}$ | 35.69±4.60$^{bB}$ |
| | 60 th day | 41.14±4.20$^{bA}$ | 37.45±4.63$^{bB}$ |
| | 90 th day | 41.96±4.25$^{bA}$ | 38.13±4.80$^{bB}$ |
| | 120 th day | 50.92±4.98$^{cA}$ | 42.21±4.80$^{cB}$ |
| **Vitamin C (mg/100 g)** | 1st day | 113.52±10.13 $^{a}$ | TNC |
| | 15 th day | 108.21±10.00 $^{b}$ | TNC |
| | 30 th day | 107.87±10.12$^{b}$ | TNC |
| | 60 th day | 107.35±10.23 $^{b}$ | TNC |
| | 90 th day | 106.57±10.00 $^{b}$ | TNC |
| | 120 th day | 104.45±10.10 $^{c}$ | TNC |

*$^{a, b, c, d, e}$: The differences between the values in the same column are statistically significant (P< .05).*
*$^{A, B,}$ The differences between the values in the same line are statistically significant (p < 0.05)*
*GPFY: Probiotic frozen yoghurt containing 20% (w/v) strawberry guava, PFY: Plain probiotic frozen yoghurt*
*TNC: Trial not conducted*

The increase in acidity measured in GPFY on the 1st (0.489% LA) and the 120th (0.721% LA) days of the storage was higher than those measured in PFY (Table 2). The relation between the increase in acidity and the pulp fortification was significant (*P*< *.05*). Güven and Karaca [39] reported that acidity increased parallel to the increase in fruit content. In their study, an increase in the acidity in ice creams was associated with probiotic cultures and pulp addition. Kosikowski [40] reported that the lactic acid % in frozen yoghurts varied between 0.31% and 1.35% (13.8 - 60.0°SH). Acidity values for GPFY and PFY in this study were compatible with previous reports [41,42].

Fat values decreased in both samples during storage. Fat values in GPFY were lower than those in PFY. Alamprese et al. [43] reported that there is a negative relationship between fat content and overrun. Overrun value in GPFY, which had lower fat values than PFY during storage, was higher. The relationship between guava pulp fortification and the decrease in fat values was significant (*P*< *.05*). Aliyev [41] reported that ice cream fat values decreased fruit fortification. Protein values decreased in GPFY and PFY samples during storage. The highest decrease was measured in GPFY. On all storage days, the decrease in protein in GPFY was greater than that of PFY. The relationship between the decrease in protein values and pulp addition was not significant (p>0.05). Some other studies also reported that protein values of frozen yoghurts increased as the fruit pulp ratio increased [41]. Bisla et al. [44], in their study on ice cream fortified with soy milk, watermelon seed milk and guava pulp (50 g), associated high protein (11.12%) and fat (7.26%) values with soy milk and watermelon seed milk. Protein values measured in this study were lower than those obtained by other researchers, whereas the fat values were close.

Vitamin C (ascorbic acid) content in strawberry guava pulp (173 mg/100 g) was affected by the pasteurization applied to the pulp (80°C/60 seconds). Vitamin C concentration of the guava pulp added to the mixture was 113.52 mg/100 g. The level of ascorbic acid conservation in the pasteurization norm applied was 66%. Vitamin C loss observed in GPFY samples during storage. Vitamin C loss rate was high on the 15th day of the storage. Associated with the increase in acidity, these values continued to decrease in the further days of the storage. Vitamin C level on the 120th day of the storage was 104.45 mg/100 g. The relationship between vitamin C loss and the increase in titratable acidity was significant (*P*< *.05*). Additionally, the relationship between the decrease in vitamin C and the cold storage was significant (*P*< *.05*). Vitamin C is a parameter used to determine the overall nutritional value of food during food storage and food processing. It has been demonstrated that other nutritional components of the food are mostly preserved when ascorbic acid is preserved after production. However, it is sensitive to various factors including ascorbic acid, pH, temperature, moisture content of the product, oxygen, light and enzymes [45].

Viscosity and hardness values in PFY samples are given in Table 2. Turgut [46] reported that microorganism species affect the viscosity; microorganisms that produce exopolysaccharides increase viscosity and *B. bifidum* increases viscosity in probiotic frozen yoghurts. It has been stated that melting resistance and smoothness increase as the viscosity increases [38]. Dry matter, viscosity and hardness values during storage in GPFY were higher than those measured in PFY. This was more evident in the later days of the storage (from the 30th day onward). The relationship between fruit pulp fortification and dry matter, viscosity and hardness values were significant in GPFY samples ($P < .05$). Also, in all samples, the relationship between the increase in storage period with dry matter, viscosity and the increase in hardness was significant ($P < .05$). Additionally, the relationship between fortification of mixture used in GPFY and PFY production with 2% (w/v) sahlep and dry matter, viscosity and hardness values were significant ($P < .05$).

### 3.3 Microbiological Properties of Probiotic Frozen Yoghurt

Microorganism counts in PY samples were; *Lb. bulgaricus* 8.61 $\log_{10}cfu^{-g}$, *Lb. acidophilus* 8.49 $\log_{10}cfu^{-g}$, *Str. Thermophilus* 8.26 $\log_{10}cfu^{-g}$ and *Bifidobacterium ssp.* 8.08 $\log_{10}$ $cfu^{-g}$, respectively. *Lb. bulgaricus, Lb. acidophilus* and *Bifidobacterium ssp.* levels in GPFY decreased until the 15th day of the storage and until the 30th day of the storage in PFY. Microorganism levels increased in both samples as storage proceeded. The increase measured after the 15th day in GPFY was higher than the increase after the 30th day in PFY. The relationship between the viability shown by the probiotic cultures in ice cream during storage and guava pulp fortification was significant ($P < .05$). The highest viability values in GPFY and PFY were determined for *Lb. bulgaricus, Lb. acidophilus* and *Bifidobacterium ssp.* in decreasing order. The largest decrease determined in the mentioned periods in PFY samples had the opposite ranking. The increase and decrease in both samples in *Lb. bulgaricus* and *Lb. acidophilus* samples were close to each other whereas the increase and decrease in *Bifidobacterium ssp.* levels were lower. *Str. thermophilus* levels decreased until the 30th day of the storage in GPFY after which it increased slightly. It decreased in PFY sample throughout the whole storage. This decrease was more evident after the 30th day (Fig. 2). The species with the highest viability in PFY samples during storage were *Lb. bulgaricus, Lb. acidophilus, Bifidobacterium ssp.* and *Str. thermophilus,* in decreasing order.

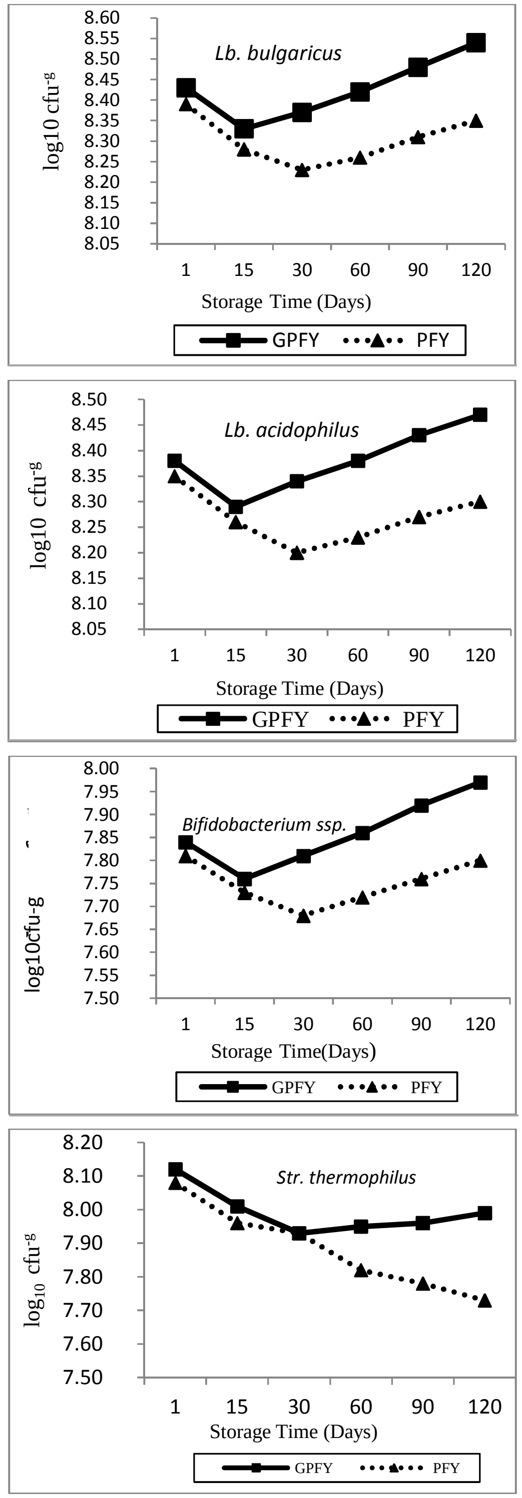


**Fig. 2. *Lb. bulgaricus, Lb. acidophilus, Bifidobacterium ssp.* and *Str. Thermophilus* counts in PY samples during storage**

In previous studies on camel milk, it has been reported that the dominant flora developed in the fermented products produced from camel milk was *Lactobacillus ssp*. [47-49]. Donkor et al. [49] reported that the viability of *Lactobacillus ssp*. was high in the presence of starch and with cold storage. In our study, higher viability values of *Lb. bulgaricus* and *L. acidophilus* compared to those of *Bifidobacterium ssp.* was associated with the starch (2.9%) added to the mixture. Viabilities of probiotic cultures during cold storage were investigated in previous studies [49,50]. Ranadheera et al. [9] reported that cocoa powder, various stabilizer (guar gum and dextrose) fortifications and high fat content (between 5-10%) were effective on the preservation of viability of probiotic microorganisms in ice cream production. Additionally, Ranadheera et al. [51] determined that *L. acidophilus* and *B. animalis subsp.* in a probiotic yogurt ice cream mixture produced from goat milk were significantly viable in the ice cream samples. Fat contents of GPFY and PFY samples stored at -20℃ for 120 days were within the limits described in the literature. There was positive and significant relation between high fat content, 2% (w/v) sahlep fortification and viability of probiotic microorganisms ($P < .05$).

### 3.4 Sensory Evaluation

In the sensory evaluation conducted during storage, GPFY samples were more appreciated in terms of textural properties (including structure-consistency, appearance and color) compared to those of PFY. In general, GPFY and PFY were more appreciated in terms of structure-consistency after the 60th day of the storage. This was associated with the increase in dry matter, viscosity, hardness and overrun during storage. The relationship between the increase in storage period and structure-consistency was found to be significant ($P < .05$). Strawberry guava pulp fortification provided an increasing taste and aroma to GPFY samples during storage which was appreciated by the panelists. There was a small but insignificant difference between GPFY and PFY samples on the 1st day of the storage, although this difference became significant in the further days of the storage. It was thought that high fat levels in samples had an effect on taste. A significant relationship was determined between the felt taste and the storage. Additionally, color change was observed in GPFY samples with pulp fortification. The panelists noted that the color change had a positive effect on the appearance of the frozen yoghurt and increased the allurement of the samples. The effect of guava fortification on the product color was more evident after the 30th day of storage.

## 4. CONCLUSIONS

Titratable acidity, dry matter, viscosity, overrun and melting resistance increased with the sahlep fortification in PFY samples. These parameters increased even more with the fruit pulp fortification. A significant relationship was found between the sahlep and fruit fortification and the physicochemical and microbiological properties of the ice creams ($P < .05$). Additionally, it was found that the relationship between high sahlep and fat contents and the fruit pulp fortification had an effect on the sustainability of the viability of probiotic microorganisms during storage. The viability observed in probiotics during storage in GPFY was higher than those in PFY samples. Microorganisms with the highest viability during storage were *Lb. bulgaricus, Lb. acidophilus, Bifidobacterium ssp.* and *Str. thermophilus*, in decreasing order. Vitamin C level was affected by the pasteurization norm applied to the mixture. Vitamin C levels showed a slight decrease in GPFY during storage, parallel to the increase in acidity. The relationship between this decrease and the cold storage was significant ($P < .05$).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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