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## The Role of Camel and Donkey Milk in Enhancing Quality Properties and *In vitro* Antioxidant and Antidiabetic Activity of Strained Yogurt

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**ABSTRACT:** With increasing emphasis on health, there has been a growing global demand for functional foods. Due to their various health and therapeutic effects, donkey and camel milk have also gained significance. The potential of these milks to be converted into products needs to be evaluated. In this study, the possibility of using a ratio of 15% and 30% camel and donkey milk together with cow milk in the production of strained yogurt has been evaluated. The physicochemical, microbiological, *in vitro* antioxidant (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Cupric Reducing Antioxidant Capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH)) and antidiabetic ( $\alpha$ -glucosidase inhibitory effect) properties of strained yogurts were evaluated during the 21 day of storage. It was observed that camel and donkey milk containing yogurts coalugum were similar to control yogurt. The addition of 30% donkey milk to cow milk significantly shortened the fermentation time ( $p<0.05$ ). Throughout the storage period, yogurts containing 15% and 30% donkey milk exhibited the highest levels of lactic acid ( $p<0.05$ ). The antioxidant activity of yogurt samples varied depending on the storage period and the method used.. Yogurt containing 30% camel milk had significantly higher *in vitro* antidiabetic activity ( $p<0.05$ ). In this study, camel and donkey milk, which are claimed to offer health benefits, was tested in yogurt manufacturing. In conclusion, these yogurt products can be included in the functional food category by evaluating various health benefits with *in vitro* and *in vivo* trials.

**Keywords:** Camel milk; Donkey milk; Yogurt; Antioxidant activity; Antidiabetic activity

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## INTRODUCTION

Functional foods have gained popularity due to their nutritional content, health-promoting characteristics, and ability to lessen the risk of numerous diseases (Aspri et al., 2017). As a result of the claimed numerous health-improving and therapeutic properties, camel milk, goat milk, and donkey milk have received increased attention (Vincenzetti et al., 2021, 2022). The OECD-FAO Agricultural Outlook 2020-2029 report states that 81% of the milk produced globally is produced by cows, 15% by buffaloes, and 4% by goats, sheep, and camels. Particularly when compared to other types of milk, donkey milk production levels are extremely low. Despite the low levels of worldwide production, donkey and camel milk are used due to their benefits for health and popularity. Aside from its therapeutic benefits, camel breeding is globally on the rise due to factors like the depletion of water resources caused by climate change. The quantitative distribution of casein and whey proteins in camel milk and cow milk differs. In the casein distribution, camel milk contains higher  $\beta$ -casein (65%) and lower  $\kappa$ -casein (3.5%) compared to cow milk (Seifu, 2023). Camel milk lacks the milk allergy-causing  $\beta$ -lactoglobulin, which is the primary whey protein found in cow milk, and contains very little  $\alpha$ -s casein. Camel milk has strong bacteriostatic qualities due to presence of several antimicrobial compound such as lysozyme, lactoperoxidase, lactoferrin, immunoglobulins, and bacteriocins (Khalesi et al., 2017). Donkey milk is chemically more comparable to human milk than cow milk. More than 60% of the total protein in donkey milk is made up of lactalbumin and lactoglobulin (Li et al., 2020). Lysozyme (13.13-15.34% of total protein) found a high concentration in donkey milk compared to cow, goat, and sheep milk (Barłowska et al., 2011). In addition to its nutritional value, donkey milk has antibacterial, antioxidant, antiviral, anti-inflammatory (Giovanna et al., 2018), immunomodulatory, and hypoallergenic properties (Aspri et al., 2017). Numerous studies have demonstrated that donkey milk significantly lowers blood sugar levels (Li et al., 2020; Akan 2021) and contains anti-aging, antioxidant, and regenerative components (Keipopele et al., 2018). Strained yogurt, also known as “Süzme” or “Torba” yogurt, is a traditional Turkish food (Şenel et al., 2011). Strained yogurt, according to Turkish legislation, is a fermented milk product with a protein concentration regulated to at least 8% (w/w) by weight, either by standardizing milk protein content or by removing the serum from the yogurt us-

ing the appropriate technology or technique. In Türkiye, strained yogurt is traditionally produced with milk from cows, sheep, goats, and buffaloes (Şenel et al., 2011) and consumed frequently.

The utilization of non-bovine milk varieties with specific nutritional features, either alone or in combination with strains of bacteria that have probiotic capabilities and/or produce biologically active metabolites, is an important alternative for the production of novel functional milk products (Yangılar, 2013). In the literature, it is seen that camel and donkey milk have attention and demand because of their potential health benefits. However, due to the nature of both camel and donkey milk, it is clear that employing these milk types directly would not produce yogurt with the desired clot hardness. For these reasons, the combination of these milk types with cow milk is a significant option in terms of better technological properties and sensory acceptability (Gomes et al., 2022). The majority of the literature focuses on the use of hydrocolloids and protein enrichment to improve camel milk coagulation ability. The number of studies focused on donkey yogurt is very rare (Salgado et al., 2021; Gomes et al., 2022). Furthermore, no research on the use of camel and donkey milk in strained yogurt has been found. In this study, strained yogurt was produced to obtain a more viscous and smooth textured product from camel and donkey milk. Another reason for choosing strained yogurt as a material is the consumption rate in Türkiye. For these reasons, in this study, the fermentation ability, physicochemical, microbiological, sensorial, and various biological properties, such as antidiabetic and antioxidant activities of strained camel and donkey yogurts mixed with cow milk were evaluated.

## MATERIAL AND METHOD

### Material

Kaya Kardeşler Camel breeding Milk production farm (Aydın, Türkiye), Ege Donkey breeding Milk production farm (Balıkesir, Türkiye), and Aydın Adnan Menderes University Faculty of Agriculture Research and Application farm (Aydın, Türkiye) provided camel milk (total dry matter:9.85%, fat:2.9% protein:2.8%), donkey milk (total dry matter:8.54%, fat:0.5% protein:1.9%), and cow milk (total dry matter:11.53%, fat:3.4% protein:3.1%) respectively. Yo-FlexExpress 1.0 (Chr.Hansen®, Hørsholm, Denmark) thermophilic yogurt culture was used. Donkey milk and camel milk were taken from 14 and 6 an-

imals, respectively. Cooled milk samples transported to the Aydın Adnan Menderes University Faculty of Agriculture Research and Application farm dairy plant at 4 °C.

## Method

### Strained yogurt production

A strained yogurt production was carried out after milk samples transferred to the dairy plant. Before milking, lactic acid-based solution was applied to the teats for 10-15 seconds and the udder was dried. After milking, the udders were dried and dipped in iodine-based solution (Dada Premix Pharmaceuticals Feed Industry and Trade Joint Stock Company, Konya, Türkiye) for a few seconds. The strained yogurt flow chart is showed in Figure 1. 3 batches (milks samples were taken one week intervals) from camel, donkey, and cow milk were heated seperately up to 95 °C for 10 minutes. After pasteurization, donkey and camel milk were added to cow milk in proportions of 15% and 30%, and mixtures were cooled to 42 °C. The yogurt starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) was mixed into the milk (16.66 mg/L) at this

temperature and incubated till the pH dropped to 4.6. Yogurts were cooled at 4 °C for a night after incubation. The next day, yogurt samples were transferred to filter cloths and filtered for 24 hours at 20-24 °C by the impact of gravity. Filtered yogurts were stored in plastic containers in refrigerator at 4 °C (Yildiz-Akgül, 2018). Strained yogurt sample codes are indicated below. Analyses were performed on the first, seventh, fourteenth, and twenty-first days of storage. Sample codes: K: control yogurt (cow milk 100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

### Physicochemical analyses

On the first day of storage, total dry matter and ash were detected using the gravimetric method, Gerber method was used to determine fat content, and nitrogen and total protein were established by micro Kjeldahl methods (AOAC, 2003).

### Fermentation kinetics

The pH changes of the inoculated milk were monitored with a pH meter every hour during fermenta-

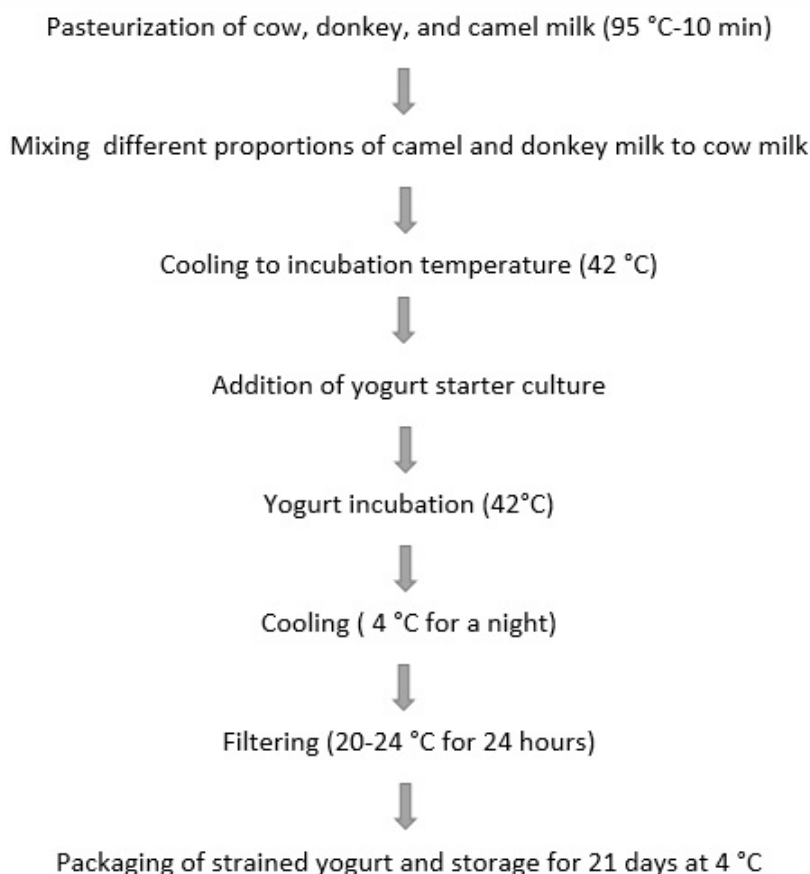


Fig. 1. Strained yogurt production flow chart

tion. Kinetic parameters were taken into account in the fermentation kinetic study.  $V_{max}$ : maximal rate of acidification (pH units/minute);  $tpH_{4.6}$ : time required to achieve pH 4.6 (hours).

### Microbiological analyses

A sample (10 g) was homogenized in 90 mL of peptone water (0.1%) under aseptic conditions. The relevant serial dilutions were then cultivated, and all analyses were performed using the pouring plate technique. For counting *L. bulgaricus* and *S. thermophilus*, MRS (de Man, Rogosa, Sharpe) agar and M17 agar were used, respectively. Colonies were counted at the end of the incubation (48-72 hours at 42 °C) under anaerobic circumstances for *L. bulgaricus* and 37°C under aerobic conditions for *S. thermophilus* (de Man et al., 1960; Terzaghi and Sandine, 1975). For yeast and mold counts, Yeast Extract Glucose Chloramphenicol (YGC) agar was employed. Colonies were counted after 3 days of incubation at 25°C for yeasts and 5 days for molds (Gonzales Fandos et al., 2000).

### Hardness

The hardness of strained yogurt samples was determined with a Material Testing Machine outfitted with a cylinder probe ( $h=12.5$  cm,  $\varnothing=6$  cm) and a 500 N force sensor (Zwick/Roel Z0.5 TH, Zwick, Germany). Immersion depth is adjusted to 25 mm (Guggisberg et al., 2009).

### Sample Preparation for Antioxidant and Antidiabetic Activity

Yogurt samples were centrifuged at 5000 x g for 20 minutes at 4 °C (Perna et al., 2015). The supernatant was filtered using filter (Whatman No. 42) before being used for antioxidant (Akan et al., 2021) and  $\alpha$ -glucosidase inhibitory activity tests.

### Antioxidant and $\alpha$ -glucosidase inhibitory activity

DPPH (2,2-Diphenyl-1-picrylhydrazil), CUP-RAC, ABTS [(2,2-azino-di-(3-) ethylbenzothialozone-sulfonic acid)] and  $\alpha$ -glucosidase inhibitory activity methods were performed with the details given in Akan (2021).

### Statistical analysis

All trials were run in triplicate (different day milks) and in parallel. One-way analysis of variance (ANOVA) SPSS version 15.0 (SPSS Inc. Chicago, Illinois) was used for statistical analysis, and differences between samples were evaluated with DUNCAN test ( $p<0.05$ ).

## RESULTS AND DISCUSSION

### Physicochemical properties and fermentation kinetics

The dry matter, protein, ash, and fat contents of strained yogurts were evaluated on the first day following production and are given in Table 1. Dry matter values of strained yogurts ranged from 20.11% to 21.65%, fat values to 7.00-7.77%, ash values to 0.56-0.70%, and protein values to 7.22-8.16%. While dry matter, fat, and protein values of the samples did not differ statistically ( $p>0.05$ ), the differences in ash values were an important ( $p<0.05$ ). The ash content of 15D and 30D samples containing donkey milk was significantly lower than the other yogurt samples ( $p<0.05$ ). Paolino et al. (2022) reported that the addition of donkey milk to Caprino cheese significantly reduced the protein, fat, and dry matter content. According to Mustafa et al. (2015), the fat, dry matter, and protein values of yogurts made with cow milk and camel milk were significantly different. Ghanbari et al. (2021) discovered a significant decrease in protein, fat, dry matter, and hardness values as the concentration of donkey milk increased, as well as an increase in acidity ( $p<0.05$ ) in donkey and cow and milk combination yogurts. In accordance with the findings, combining 25% and 50% donkey milk with cow milk

**Table 1.** Physicochemical analysis results of strained yogurt samples (%)

Sample/Parameter	Dry matter (%)	Fat (%)	Ash (%)	Protein (%)
K	21.23±1.23 <sup>a</sup>	7.00±0.63 <sup>a</sup>	0.70±0.02 <sup>c</sup>	8.85±2.15 <sup>a</sup>
15C	21.32±1.86 <sup>a</sup>	7.50±0.54 <sup>a</sup>	0.67±0.01 <sup>c</sup>	7.30±0.79 <sup>a</sup>
30C	20.11±2.22 <sup>a</sup>	7.17±0.40 <sup>a</sup>	0.67±0.02 <sup>c</sup>	9.31±2.37 <sup>a</sup>
15D	21.12±1.69 <sup>a</sup>	7.77±0.75 <sup>a</sup>	0.60±0.1 <sup>b</sup>	7.52±1.25 <sup>a</sup>
30D	21.65±1.24 <sup>a</sup>	7.66±0.51 <sup>a</sup>	0.56±.01 <sup>a</sup>	7.22±0.77 <sup>a</sup>

K: cow milk (100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

<sup>a-c</sup>Mean within the same column marked with different letters differ significantly ( $p<0.05$ ).



is acceptable in terms of physicochemical, textural, and sensory qualities (Ghanbari et al., 2021).

Fermentation times of yogurt samples varied between 263.33 and 293.33 minutes. 30D and 30C samples had the shortest and longest fermentation times, respectively (Table 2). When camel milk is fermented with starter cultures, it has gels with a more aqueous/smooth consistency and a weaker texture than cow milk yogurt (Agrawal et al., 2007). In the 15C and 30C samples clot was properly formed in our investigation, but their fermentation periods were longer than the control sample ( $p < 0.05$ ). The reasons for the longer fermentation time could be the lack of  $\beta$ -lactoglobulin, low kappa casein concentration, the content of heat-stable serum proteins (Desouky et al., 2013), the relative distribution of casein fractions (Al Kanhal, 2010) and the size of casein micelles being larger than in cow's milk (Kamal et al., 2017). However, we discovered that combining 15% and 30% camel milk with cow milk is appropriate for coagulum formation in yogurt production. Berhe et al. (2017) indicated that camel milk can be fermented with several commercial cultures; however, the fermentation duration is too long. Besides, in this study, the fermentation times of donkey yogurts were shorter than

camel yogurts. Especially, the fermentation time of the 30D sample showed a statistically difference from the control and camel yogurts ( $p < 0.05$ ). According to Gomes et al. (2022), the fermentation duration of donkey yogurt with cow milk was 240 seconds, and there was no coagulum problem in yogurts. Our study results support this research.

### pH and titratable acidity

For 21 days, the pH and lactic acid values of strained yogurts ranged from 4.02-4.29 and 1.30-1.56%, respectively (Table 3). There was similarity between the pH values of the yogurts at the first day of storage ( $p > 0.05$ ). The pH values of the K and 30C samples were higher than the other samples on the 21st day ( $p < 0.05$ ). At the end of the storage period, the pH values of the control and camel yogurts were substantially higher than on the 14th day ( $p < 0.05$ ). This could be due to an increase in yeast numbers at the end of storage, as well as their usage of lactic acid as a carbon source. Despite the increase in yeast counts, the pH values of 15D and 30D samples were not substantially different on the 21st day with regard to the 14th day. This is due to the antibacterial features of donkey milk. The highest acidity levels were identified during

**Table 2.** Fermentation kinetic parameters and yield (%)

Sample/Parameter	Vmax ( $10^{-3}$ pH unit/second)	Time reach to pH 4.6 (second)	Yield (%)
K	7,35±0,06 <sup>c</sup>	275,00±5,00 <sup>b</sup>	35.40±0.72 <sup>c</sup>
15C	7,02±0,04 <sup>b</sup>	283,33±2,88 <sup>c</sup>	32.00±0.00 <sup>bc</sup>
30C	6,54±0,15 <sup>a</sup>	293,33±2,88 <sup>d</sup>	28.53±4.71 <sup>b</sup>
15D	7,39±0,11 <sup>c</sup>	276,66±2,88 <sup>b</sup>	27.67±3.93 <sup>ab</sup>
30D	8,01±0,09 <sup>d</sup>	263,33±2,88 <sup>a</sup>	22.01±2.75 <sup>a</sup>

K: cow milk (100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

a-d Mean within the same column marked with different letters differ significantly ( $p < 0.05$ ).

**Table 3.** pH and titratable acidity values of strained yogurt samples

pH/Sample	K	15C	30C	15D	30D
1	4.29±0.04 <sup>aZ</sup>	4.25±0.03 <sup>aT</sup>	4.27±0.02 <sup>aZ</sup>	4.24±0.01 <sup>aY</sup>	4.24±0.01 <sup>aX</sup>
7	4.07±0.01 <sup>aX</sup>	4.06±0.01 <sup>aY</sup>	4.06±0.01 <sup>bX</sup>	4.04±0.01 <sup>bX</sup>	4.04±0.02 <sup>abY</sup>
Storage day	14	4.02±0.03 <sup>abX</sup>	3.98±0.01 <sup>bX</sup>	4.07±0.01 <sup>bcX</sup>	4.09±0.04 <sup>cX</sup>
	21	4.15±0.00 <sup>bY</sup>	4.10±0.01 <sup>aZ</sup>	4.14±0.01 <sup>bY</sup>	4.11±0.05 <sup>cY</sup>
	4.11±0.01 <sup>aY</sup>				
Lactic acid (%) / Sample	K	15C	30C	15D	30D
1	1.46±0.03 <sup>bZ</sup>	1.37±0.07 <sup>abX</sup>	1.30±0.10 <sup>aX</sup>	1.48±0.01 <sup>bY</sup>	1.48±0.04 <sup>bXY</sup>
7	1.43±0.03 <sup>bYZ</sup>	1.41±0.07 <sup>abX</sup>	1.31±0.05 <sup>aX</sup>	1.48±0.08 <sup>bcY</sup>	1.56±0.04 <sup>cY</sup>
Storage day	14	1.38±0.05 <sup>aXY</sup>	1.41±0.06 <sup>aX</sup>	1.39±0.10 <sup>aX</sup>	1.52±0.03 <sup>aY</sup>
	21	1.33±0.03 <sup>abX</sup>	1.32±0.05 <sup>abX</sup>	1.37±0.10 <sup>abX</sup>	1.30±0.04 <sup>aX</sup>
					1.43±0.03 <sup>bXY</sup>

K: cow milk (100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

a-d Mean within the same row marked with different letters differ significantly ( $p < 0.05$ ).

X,Y,Z,T Mean within the same column marked with different letters differ significantly ( $p < 0.05$ ).

storage in 15D and 30D samples ( $p < 0.05$ ). According to Wang et al. (2023), the acidity level of camel yogurt was lower than cow yogurt. This was related to the amount of living bacteria in the product. Because camel milk was combined with cow milk in our investigation, our results did not match those of Wang et al. (2023). Mustafa et al. (2015) discovered that yogurts with more camel milk had higher lactic acid levels and longer fermentation times than yogurts with cow milk, but there was no significant variance in pH values ( $p > 0.05$ ). Kamal-Eldin et al. (2020) determined no significant variation in pH values between yogurts that are produced with camel milk and cow milk (ratios of 10%, 20%, 40%, and 60%) ( $p > 0.05$ ). In our investigation, the pH value of the 30C sample was higher than the 15C sample during storage, with the exception of the first day ( $p < 0.05$ ). This revealed that the watery coagulation of camel milk was caused by the structure and concentration of camel milk proteins rather than the acidification rate.

### Microbiological properties

Yogurt bacteria, and yeast-mold counts were evaluated in yogurts 21-day storage period and are shown in Table 4. *S. thermophilus* was found to be more prevalent than *Lactobacillus bulgaricus* in the yogurt medium and to be more than 8 log CFU/g in all yogurts during the storage period. Donkey and camel milk samples had a higher *S. thermophilus* count on the first day of storage than the control yogurt ( $p < 0.05$ ). At the end of the storage, the lowest and highest *Lac-*

*tococcus* spp. counts were determined in the 30C and 15C samples, respectively. In all samples, the number of *L. bulgaricus* varied between 3.76-6.71 log CFU/g. The number of *L. bulgaricus* in the control sample remained low on the first day of storage as regards the other samples ( $p < 0.05$ ). The count of *L. bulgaricus* in the 15C and 30C yogurt samples was higher on the first day of storage than other yogurt samples ( $p < 0.05$ ). The 30D sample had a greater *L. bulgaricus* number than the 15D sample during storage ( $p < 0.05$ ). The count of *L. bulgaricus* in all yogurt samples fell significantly on the 21st day compared to the beginning ( $p < 0.05$ ). According to Eissa et al. (2011), the count of *L. bulgaricus* and *S. thermophilus* in cow yogurt was higher than in camel yogurt during storage. The *L. bulgaricus* and *S. thermophilus* levels in control yogurt were lowest on the 1st day of storage in our study.

The yeast-mold count ranged from 1.10 to 2.04 log CFU/g in the 1st day of storage, but decrease in all samples during the next few days, reaching a range of 5.83-6.3 log CFU/g 21-days of storage. While the yeast count in the control sample was the highest at the start of storage ( $p < 0.05$ ), the yeast counts in the other yogurt samples decreased during storage (7, 14, and 21 days) and did not differ from the control sample. This case demonstrated that using donkey and camel milk in strained yogurts had no inhibitory effect on yeast development during preservation. Furthermore, it is possible that the number of yeast was decreased as a result of contamination of yogurt samples during

**Table 4.** Microbiological properties of strained yogurt samples (log CFU/g)

<i>L. bulgaricus</i> /Sample	K	15C	30C	15D	30D
1	4.26±0.24 <sup>aXY</sup>	6.71±0.17 <sup>dZ</sup>	6.63±0.26 <sup>dZ</sup>	4.77±0.07 <sup>bY</sup>	6.10±0.31 <sup>cY</sup>
7	4.96±0.91 <sup>aY</sup>	6.49±0.05 <sup>bZ</sup>	5.79±0.14 <sup>bY</sup>	4.68±0.11 <sup>aY</sup>	6.09±0.02 <sup>bY</sup>
14	4.56±0.33 <sup>aXY</sup>	5.70±0.20 <sup>bY</sup>	5.50±0.07 <sup>bY</sup>	4.87±0.30 <sup>aY</sup>	5.79±0.04 <sup>bY</sup>
Storage day 21	3.84±0.30 <sup>abX</sup>	4.92±0.70 <sup>cX</sup>	3.75±0.43 <sup>aX</sup>	3.76±0.11 <sup>aX</sup>	4.65±0.46 <sup>bcX</sup>
<i>S. thermophilus</i> /Sample	K	15C	30C	15D	30D
1	8.63±0.54 <sup>aX</sup>	9.02±0.04 <sup>abX</sup>	9.17±0.20 <sup>abY</sup>	9.44±0.12 <sup>bY</sup>	8.99±0.19 <sup>abX</sup>
7	9.06±0.07 <sup>abXY</sup>	9.34±0.35 <sup>bXY</sup>	8.86±0.09 <sup>aX</sup>	8.97±0.10 <sup>aX</sup>	8.90±0.07 <sup>aX</sup>
Storage day 14	9.03±0.19 <sup>aXY</sup>	9.20±0.02 <sup>aXY</sup>	9.27±0.11 <sup>abY</sup>	9.47±0.16 <sup>bY</sup>	9.29±0.10 <sup>abY</sup>
21	9.49±0.27 <sup>abY</sup>	9.51±0.17 <sup>bY</sup>	9.19±0.04 <sup>aY</sup>	9.39±0.05 <sup>abY</sup>	9.31±0.14 <sup>abY</sup>
Yeast-mould/ Sample	K	15C	30C	15D	30D
1	2.04±0.59 <sup>bX</sup>	1.10±0.17 <sup>aX</sup>	1.25±0.24 <sup>aX</sup>	1.30±0.00 <sup>aX</sup>	1.59±0.53 <sup>abX</sup>
7	3.48±0.21 <sup>bY</sup>	4.14±0.25 <sup>aY</sup>	2.38±0.12 <sup>aY</sup>	3.79±0.21 <sup>aX</sup>	3.42±0.51 <sup>aX</sup>
Storage day 14	4.80±0.23 <sup>dZ</sup>	4.15±0.26 <sup>bcZ</sup>	4.33±0.12 <sup>cdZ</sup>	3.79±0.22 <sup>abY</sup>	3.42±0.51 <sup>aY</sup>
21	6.01±0.23 <sup>aT</sup>	6.18±0.72 <sup>aT</sup>	6.33±0.20 <sup>aT</sup>	5.83±0.18 <sup>aZ</sup>	6.09±0.53 <sup>aZ</sup>

K: cow milk (100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

<sup>a-d</sup> Mean within the same row marked with different letters differ significantly ( $p < 0.05$ ).

X,Y,Z,T Mean within the same coloumn marked with different letters differ significantly ( $p < 0.05$ )

the storage. Eissa et al. (2011) stated that the count of yeasts in camel and cow milk yogurts decreased constantly throughout storage, which is similar to our findings. During the 28-day storage period, the lactic acid bacteria count of donkey milk yogurt was higher than that of cow milk yogurt ( $p<0.05$ ), according to Salgado et al. (2021). In our investigation, similar findings were achieved in 15D and 30D samples on the first day of storage.

### Hardness

The texture of the gel is a key parameter for assessing the quality of set-style yogurt. Table 5 shows the hardness values of strained yogurt samples. The 15D sample has the highest hardness value at the beginning and end of storage. While the hardness values of strained yogurts did not differ statistically ( $p>0.05$ ) at the start of storage, a significant difference was found on the 21st day ( $p<0.05$ ). From the seventh day of storage, strained yogurts containing donkey milk had higher hardness values than strained yogurts containing camel milk ( $p<0.05$ ). The hardness values of the 30C sample were lower than those of the control sample on the seventh day of storage ( $p<0.05$ ). Similar to our findings, Kamal-Eldin et al. (2020) found that decreasing the percentage of camel milk to cow milk enhanced the hardness value of yogurt. Camel milk and donkey milk have different protein compositions than cow milk (Atwaa et al., 2020). Amino acids and protein structure are two factors that influence protein water retention. Simultaneously, syneresis in yogurt varies depending on the protein composition of milk (Ibrahim and Hhalifa, 2015). According to our findings, discrepancies in the hardness value of cow, camel, and donkey milk strained yogurt can be attributed to variances in protein structures, water holding capacity, and syneresis sensitivity. Furthermore, the declining trend in camel milk yogurt hardness may be attributed to biochemical, enzymatic, or acid production alterations that promote syneresis and weaken the yogurt texture (Yadav et al., 2007).

### Antioxidant activity

Yogurt has an antioxidant effect due to the release of small-molecule peptides and amino acids with antioxidant activity during fermentation (Wang et al., 2023). In general, the antioxidant activity of fermented dairy products is affected by starter culture type and amount, enzyme types, and protein hydrolysis (Yilmaz-Ersan et al., 2016).

In this study, the antioxidant activity was calculated using the ABTS, CUPRAC, and DPPH techniques, and the results were presented in  $\mu\text{M}$  trolox (Figure 2a, 2b, 2c). Antioxidant activity of samples in the ABTS method ranged from 0-199.13, 209.72-507.50 in the CUPRAC method, and 0-98.90  $\mu\text{M}$  trolox in the DPPH method. 30D sample demonstrated the highest antioxidant activity on the first day of storage using the ABTS technique. Except for the beginning of storage, there was a statistical similarity in antioxidant activity values between samples ( $p<0.05$ ). Soleymanzadeh et al. (2016) discovered that the antioxidant activity by the ABTS test of fermented cow and camel milk increased over time. Dharmisthabet et al. (2023) reported that antioxidative peptides were released during the camel milk fermentation. According to Perna et al. (2015), probiotic donkey yogurt showed better antioxidant activity (ABTS technique) than cow yogurt, and the antioxidant activity decreased with storage ( $p<0.05$ ). The K sample showed the highest antioxidant activity on the first day, according to the CUPRAC technique ( $p<0.05$ ). Other than the 21st day of storage, the antioxidant activity of the samples differed ( $p<0.05$ ). Only on the 14th day, camel and donkey milk yogurt samples demonstrated stronger antioxidant activity than control yogurt ( $p<0.05$ ). The maximum activity was discovered in the 30C sample on the first day, whereas it was detected in the K sample on the 21st day ( $p<0.05$ ), according to the DPPH results. On the 14th day of storage, the DPPH method was unable to detect antioxidant activity in the 15D and 30D samples. Except for the 15C sample, the an-

**Table 5.** Hardness (N) values of strained yogurt samples

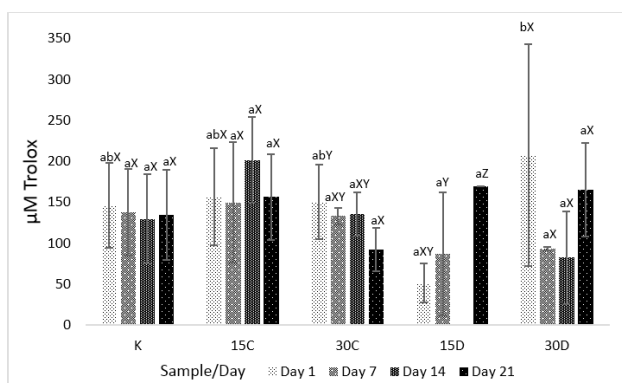
Hardness/Sample	K	15C	30C	15D	30D
<b>1</b>	13.36±0.66 <sup>aX</sup>	17.60±3.72 <sup>aY</sup>	10.41±1.78 <sup>aX</sup>	18.70±10.40 <sup>aX</sup>	16.13±5.50 <sup>aX</sup>
<b>7</b>	12.64±4.15 <sup>abX</sup>	10.73±0.55 <sup>abX</sup>	7.28±2.38 <sup>aX</sup>	19.90±9.44 <sup>bX</sup>	16.46±4.47 <sup>abX</sup>
<b>14</b>	13.65±4.85 <sup>abX</sup>	9.18±2.05 <sup>aX</sup>	6.88±2.21 <sup>aX</sup>	17.33±2.25 <sup>bX</sup>	16.80±5.80 <sup>bX</sup>
<b>21</b>	12.30±1.08 <sup>abX</sup>	12.45±4.37 <sup>abXY</sup>	6.71±0.98 <sup>aX</sup>	14.60±6.85 <sup>bX</sup>	14.03±3.15 <sup>abX</sup>

K: cow milk (100%); 15C: cow milk: camel milk; 85:15 (v/v); 30C: cow milk: camel milk; 70:30 (v/v); 15D: cow milk: donkey milk; 85:15 (v/v); 30D: cow milk: donkey milk; 70:30 (v/v).

<sup>a-d</sup> Mean within the same row marked with different letters differ significantly ( $p<0.05$ ).

X,Y,Z,T Mean within the same coloumn marked with different letters differ significantly ( $p<0.05$ ).

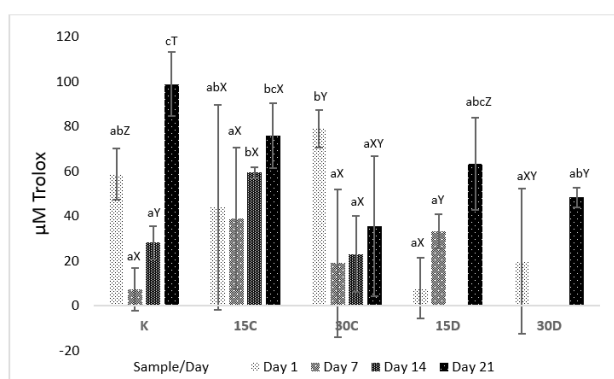




**Fig. 2a.** Antioxidant activity analyses from ABTS method

a-d Different letters on bars represent significant differences among samples same storage day ( $p < 0.05$ ).

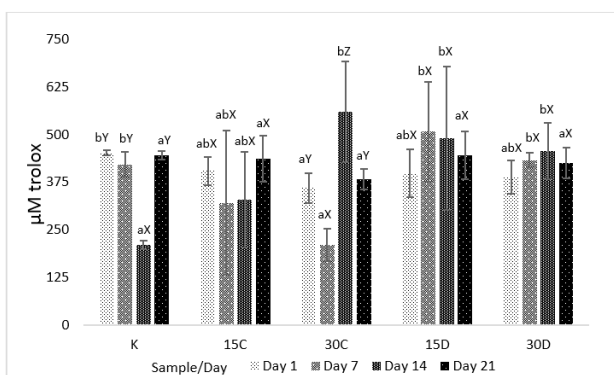
X,Y,Z,T Different letters on bars represent significant differences during the storage ( $p < 0.05$ ).



**Fig. 2b.** Antioxidant activity analyses from DPPH method

a-d Different letters on bars represent significant differences among samples same storage day ( $p < 0.05$ ).

X,Y,Z,T Different letters on bars represent significant differences during the storage ( $p < 0.05$ ).



**Fig. 2c.** Antioxidant activity analyses from CUPRAC method

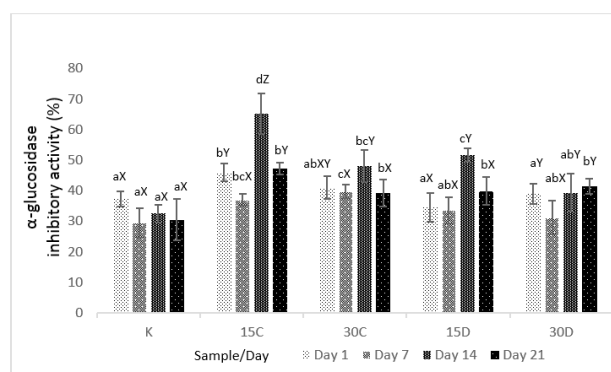
a-d Different letters on bars represent significant differences among samples same storage day ( $p < 0.05$ ).

X,Y,Z,T Different letters on bars represent significant differences during the storage ( $p < 0.05$ ).

tioxidant activity of all samples changed significantly during the storage ( $p < 0.05$ ). Cow milk kefir has stronger DPPH inhibitory action than donkey milk kefir ( $p < 0.05$ ) (Yirmibeşoğlu and Tefon Öztürk, 2020). According to Wang et al. (2023), the antioxidant activity of camel milk yogurt measured using the DPPH technique was substantially higher than goat and cow milk yogurt ( $p < 0.05$ ). In this study, depending on the method, antioxidant activity results vary. While the DPPH test measures the activity of hydrophilic molecules, the ABTS and CUPRAC methods measure the antioxidant activity of both hydrophobic and hydrophilic compounds (Akan, 2021). Similarly, discrepancies in lipophilic and hydrophilic antioxidant systems may be caused by differences in radical capture mechanisms and the sensitivity of analysis methodologies (Solaymenzadeh et al., 2016). Tak et al. (2018) and Solaymenzadeh et al. (2016) found that antioxidant activity (DPPH method) in fermented camel products was lower than the ABTS method. At  $p < 0.05$  level, we found a positive correlation ( $r = 0.54$ ) between the DPPH and ABTS techniques. Savaş and Akan (2021) discovered a positive high-level correlation ( $r = 0.682$ ) between CUPRAC and DPPH techniques that result in probiotic yogurts.

### Antidiabetic activity

During the storage period, the  $\alpha$ -glucosidase inhibitory activity of the yogurts ranged from 29.48 to 65.23% (Figure 3). Except for the 7th day, the 15C sample indicated the highest  $\alpha$ -glucosidase inhibitory activity over the storage period ( $p < 0.05$ ). In general, the control yogurt had the least  $\alpha$ -glucosidase



**Fig. 3.**  $\alpha$ -glucosidase inhibitory activities of yogurt samples

a-d Different letters on bars represent significant differences among samples same storage day ( $p < 0.05$ ).

X,Y,Z,T Different letters on bars represent significant differences during the storage ( $p < 0.05$ ).

inhibitory activity. Samples containing 15% camel milk demonstrated higher activity during storage than samples containing 15% donkey milk. Yogurts containing camel milk show much stronger *in vitro* anti-diabetic activity than donkey yogurts. Furthermore, donkey yogurts had higher activity than control yogurt on the 7th, 14th, and 21st days of storage. Camel, donkey, and cow milk yogurts exhibited the strongest *in vitro* antidiabetic activity in our investigation. Shori and Baba (2014) found that fermented camel milk has more  $\alpha$ -glucosidase inhibitory values than fermented cow milk. Shukla et al., (2023) reported that the  $\alpha$ -glucosidase inhibitory activity of fermented camel milk was 64.45. Several studies have demonstrated the significant impacts of camel milk on diabetes (Anwar et al., 2022; AlKurd et al., 2022). The exceptional antidiabetic activity of camel milk is mainly attributed to peptides generated during the fermentation and storage processes (Akan, 2021; Mahmoudi et al., 2022).

## CONCLUSION

In this study, the use of various proportions of camel and donkey milk with cow milk in the production of strained yogurt was investigated. The general composition, hardness values, count of yogurt bacteria and antioxidant activity of yogurts vary depending on the amount of camel and donkey milk used. It was

observed that camel and donkey milk yoghurt curds were similar to cow milk. Addition of 30% donkey milk to cow milk significantly shortened the fermentation time ( $p < 0.05$ ). Using donkey and camel milk in strained yoghurts did not prevent yeast growth during storage. It was observed that yoghurts containing camel milk had higher *in vitro* antidiabetic activity than donkey yoghurts and control yoghurts. Technologically, the usage of 15% and 30% ratios of camel and donkey milk to cow milk is appropriate in the manufacturing of strained yogurt. According to the findings, camel and donkey milk, which are claimed to offer health benefits, could be tested in yogurt manufacturing. These yogurt products can be included in the functional food category by evaluating various health benefits with *in vitro* and *in vivo* trials.

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## CONFLICT OF INTEREST

None declared.

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