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Baniasadi, Mehdi

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Comparative antioxidant potential of kefir and yogurt of bovine and non-bovine origins

Mehdi Baniasadi[1], Maryam Azizkhani[2*], Per Erik Joakim Saris[3], Fahimeh Tooryan[4]

[1]M.Sc.,Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Aftab 24 St., Imam Khomeini Av., Amol, Iran. Email: mehdi_food@yahoo.com

[2]Ph.D., Associate Professor, Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Aftab 24 St., Haraz Av. Amol, Iran. Email: azizkhani.maryam@gmail.com

[3]Ph.D., Full Professor, Department of Microbiology, University of Helsinki, Helsinki, Finland. Email: Per.Saris@helsinki.fi

[4]Ph.D., Assistant Professor, Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Aftab 24 St., Haraz Av. Amol, Iran. Email: f.tooryan@gmail.com

*Corresponding author:

Maryam Azizkhani, Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Aftab 24 St., Haraz Av. Amol, Iran, P.O.: 46186-49767, Email: azizkhani.maryam@gmail.com; ORCID: 0000-0001-5366-1660

Abstract

The aim of this study was to compare the antioxidant potential of the yogurt and kefir produced from ewe, camel, goat, and cow milk. The antioxidant activity of the samples was assessed by measuring total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical reducing capacity during 20-day storage at 4 ºC. Kefir and yogurt prepared from ewe and camel milk had significantly higher antioxidative potential than samples made from goat and cow milk ($P < 0.05$). Ewe kefir (74.55-80.11 mg GAE 100 mL^{-1}) showed the highest TPC followed by cow kefir (65-73.15 mg GAE 100 mL^{-1}), camel kefir (61.2-69.91 mg GAE 100 mL^{-1}) and goat kefir (58.31-73.5 mg GAE 100 mL^{-1}) ($P < 0.05$). Camel yogurt possesses the highest TPC (56.5-68.25 mg GAE 100 mL^{-1}) followed by ewe (40.32-46.5 mg GAE 100 mL^{-1}), cow (29.5-35.5 mg GAE 100 mL^{-1}) and goat (20.03-26.85 mg GAE 100 mL^{-1}) yogurt ($P < 0.05$). According to DPPH, FRAP, and ABTS results, the antioxidant activity of samples was as follows in descending order: ewe kefir, camel kefir, ewe yogurt, camel yogurt, cow kefir, goat kefir, goat yogurt, cow yogurt.

KEYWORDS: antioxidant activity, kefir, milk, yogurt

Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid
ºC	degree Celsius
cm	centimeter
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
h	hour
M	molar
min	minute
mL	milliliter
µm	micrometer
nm	nanometer
rpm	round per minute
TPC	total phenolic content
v/v	volume/volume

w/v weight/volume

Practical applications

The oxidative stress and damage due to production of free radicals and reactive oxygen species in food and body plays a considerable pathological role in health risk and human diseases such as cancer. It is obvious that diets containing high amount of natural antioxidants are helpful to reduce the incidence of oxidative stress related diseases and cancer. Fermented dairy products possess antioxidative potential (Zulueta et al., 2009) and we assumed that this activity varies depending on origins of milk, the composition of milk and also fermenting microorganisms. The awareness of consumers about the harmful effects of synthetic antioxidants on health and the advantages of using functional natural foods is increasing worldwide. Determining the antioxidative properties of the two most-consumed fermented dairy products, kefir and yogurt, would be useful to support the healthful and biofunctional claims about them to the consumers.

1 INTRODUCTION

Milk is known as a nutritionally valuable food that contains a wide range of micro and macronutrients and considered as the main source of energy for mammalian infants. Furthermore, it has been found that enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic (lactoferrin, casein, α-LA, β-LG, tryptophan, cysteine, tyrosine, lysine, carotenoids, uric acid, vitamins A, C, and E) antioxidants are naturally present in the milk of different mammalian species. Therefore, it seems that milk has health-beneficial and functional effects against the production of reactive oxygen species and oxygen-free radicals which otherwise results in oxidative stress (Zulueta et al., 2009).

Among the fermented milk products, kefir and yogurt are the most popular. Yogurt is produced from bacterial (yogurt cultures) fermentation of milk. Kefir drink is fermented milk produced from kefir grains that has originated from the Caucasus, Eastern Europe, and Russia. Kefir grains are gelatinous irregularly masses with white or light yellow color and consisted of a symbiotic mixture of lactic and acetic acid bacteria (*Lactobacillus helveticus, L. brevis, L. kefir,* and *Leuconostoc mesenteroides*), several genera of yeasts (*Kluyveromyces lactis, K. marxianus,* and *Pichia fermentans*), and mycelial fungi aggregated in a polysaccharide matrix named kefiran (Yilmaz, Ozcan Yilsay, & Akpinar Bayizit, 2006). Therefore, kefir is different from yogurt and other types of fermented milk products as it is produced as the result of the metabolic activity of a wide range of microorganisms of microflora of kefir grains. There are studies that showed the microorganisms of the yogurt starter culture produce lactic acid and natural bioactive compounds (like peptides, amino acids, and organic acids) and antibiotics (such as bacteriocins) during fermentation of cow milk (Leite et al., 2013). Also, it has been reported that kefir has beneficial effects on human nutrition and health, such as improving the function of the immune system and digestive organs, helping the treatment of blood hypertension, allergies, metabolic defects and heart diseases (Cenesiz, Devrim, Kamber, & Sozmen, 2008).

In several works, the antioxidant and antimicrobial potential of milk and milk products (fermented and non-fermented) were indicated (de Lima et al., 2018; Gamba et al., 2016; Rosa et al., 2017; Turkmen, 2017; Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, & Sahin, 2016, 2018) but little information is found about the antioxidative properties of kefir and yogurt produced from different types of milk. It is assumed that fermented dairy products possess different antioxidant capacity based on their milk source, starter culture, and shelf-life; therefore, the objective of this study was to I) determine total phenolic content, and II) compare the antioxidant potential of the yogurt and kefir produced from ewe, camel, goat, and cow milk during cold storage at 4 ºC.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

All the chemicals and reagents (sodium hydroxide, methanol, pH meter buffers, sodium carbonate, copper sulphate, Folin–Ciocalteu reagent, gallic acid, potassium sodium tartrate tetrahydrate, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, potassium persulphate, ferrous sulphate, Iron(III) chloride, ferric tripyridyl triazin) used in this study were purchased from Merck (Germany).

2.2 Preparation of kefir and yogurt inoculums

Raw cow, ewe, and goat milk were obtained from the dairy farm of Bandpei (Mazandaran, Iran) and camel milk was purchased from a camel farm in Kalaleh (Golestan, Iran). Commercial starter culture (containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) as direct vat set culture purchased from Danisco (Denmark) was used for yogurt production. Traditional kefir grains were obtained from rural areas of Semnan (Semnan, Iran). In order to recover, the kefir grains were transferred into pasteurized low-fat cow milk (0.45% fat content) and incubated at 25 ± 1 °C for 24 h and this step was repeated for 7 consecutive days. After this 7-day period, the kefir grains were filtered to separate the milk curd and washed with sterile distilled water 3 times. Then, the grains were inoculated into pasteurized cow milk and kept at 25 ± 1 °C until used.

2.3 Measuring the total solid, fat, protein, and lactose content of milk samples

120 The fat and protein content of milk samples was measured by the Gerber method (Kleyn, Lynch,
121 Barbano, Bloom, & Mitchell, 2001) and the Kjeldahl method (Tremblay, Laporte, Leonil, Dupont, &
122 Paquin, 2003), respectively. Total solid and lactose contents were determined according to Boci et al.
123 and Sharma et al. respectively (Boci, Bardhi, & Cakraj, 2013; Sharma, Rajput, Dogra, & Tomar, 2009).
124
125 **2.4 Kefir and yogurt production**
126 Milk was heated to 90 ± 1 °C for 10 min in the hot water bath and cooled to the temperature appropriate
127 for inoculation (25 °C for kefir and 43 °C for yogurt). Kefir samples were prepared (in 250 mL glass
128 bottles) by inoculating kefir grains (5% v/v) to each individual milk and incubating (Memmert Incubator
129 400, Switzerland) at 25 °C for 20 h. The probiotic yogurt samples were produced by mixing milk samples
130 and starter culture (2% v/v) followed by incubation at 43-45 °C until reaching a pH 4.6±0.1. At the end
131 of the fermentation step, the kefir samples were filtered through a sterile metal sieve (1.5 mm pore size)
132 in order to separate the kefir grains and then filled into 250 mL glass bottles with plastic lid (Figure 1).
133 Yogurt samples were stored in glass jars with plastic lid.
134 Kefir and yogurt samples were kept at 4±1°C until analysis. The samples were analyzed on the 1^{st}, 5^{th},
135 10^{th}, 15^{th}, and 20^{th} days of storage.
136
137 **Figure 1**
138
139 **2.5 Preparation of the kefir and yogurt extracts for the assays**
140 Two grams of the kefir and yogurt samples were mixed with 20 mL of extracting solvent
141 (methanol/water, 70:30 v/v) and blended thoroughly on a magnetic stirrer (model RSM-03-10K, Phoenix,

Germany) at 20±1 °C for 4 h in a dark place. Then it was centrifuged (model Z206A, Hermle, Germany) at 3,000 rpm for 12 min at 4 °C and filtered through Whatman™ 12.5 cm Grade 2 cellulose qualitative filter paper (Diameter: 12.5 cm, Pore Size: 8 µm). The obtained supernatants were used to determine pH, total phenolic contents and antioxidant activity by DPPH, FRAP, and ABTS assay (Yilmaz-Ersan et al., 2016).

2.6 pH measurement

The pH-values of the filtered kefir and yogurt supernatants were measured with a pH meter model 913 (Metrohm, Switzerland). The pH meter was calibrated by pH 4.00 and 7.00 standard buffers.

2.7 Determination of total phenolic content in kefir and yogurt samples

The total phenolic content (TPC) of the samples was measured by applying the Folin–Ciocalteu method (Şahin, Aybastıer, & Işık, 2013). The solutions were prepared as described below: solution A: 2% of aqueous Na_2CO_3 in NaOH (0.1 M); solution B: 0.5% of aqueous $CuSO_4$ in 1% $NaKC_4H_4O_6$ solution; solution C: a mixture of 50 mL solution A and 1 mL solution B which was prepared freshly; Folin–Ciocalteu reagent was prepared by diluting its stock solution with H_2O at a ratio of 1:3 (v/v). In order to perform the assay, 0.1 mL of kefir or yogurt extract was mixed with 1.9 mL of H_2O and 2.5 mL of solution C and the mixture was kept in ambient temperature for 10 min. Then, 0.25 mL of Folin–Ciocalteu reagent was added and incubated at room temperature for 30 min to stabilize the blue color. The absorbance of the solution was measured by spectrophotometer (model Lambda 365, Perkin Elmer, USA) at 750 nm. The standard calibration curve was obtained using several concentrations of gallic acid. TPC was calculated from the plotted standard curve and expressed as mg of gallic acid equivalent (GAE) per 100 mL of sample.

2.8 Diphenyl picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant capacity of kefir and yogurt samples was assessed through 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) (Şahin, Işık, Aybastıer, & Demir, 2012; Yilmaz-Ersan et al., 2018). Briefly, 0.25 mL of kefir or yogurt extract was added to 0.18 mL of DPPH reagent (10^{-3} M of stock solution) in a tube and mixed. Then, methanol was added to obtain the final volume of 3 mL. The tube was kept in the dark for 30 min and the absorbance was read using a spectrophotometer (model Lambda 365, Perkin Elmer, USA) at 517 nm against a blank. The standard curve was prepared using different concentrations of Trolox (as the standard solution for calibration), and the results were expressed as mg of Trolox Equivalents (TE) per 100 mL of sample and the percentage of antioxidant activity was calculated using the following formula Eq. (1):

$$\text{Radical Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

which $A_{control}$ was the absorbance of control and A_{sample} was the absorbance of the sample contained kefir or yogurt extract.

2.9 Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) assay was performed to compare the antioxidative capacity of kefir and yogurt samples (Benzie & Strain, 1996). In this method, iron acts as a redox agent so the technique is designed upon the reduction of Fe^{3+}-TPTZ (ferric tripyridyl triazine) to Fe^{2+}-TPTZ by the antioxidants. A blue color appears as the result of this reduction which is quantified by measuring

the absorbance at 593 nm. The working solution (FRAP reagent) was prepared by blending 10 volumes of acetate buffer (1.0 M, pH 3.6), 1 volume of TPTZ (10 mM in 40 mM HCl) and 1 volume of $FeCl_3$ (20 mM). In a dry test tube, 0.25 mL of kefir or yogurt extract and 2.75 mL of the FRAP reagent were mixed and kept at 37 °C for 30 min. Then, the solution was centrifuged at 3000 rpm for 10 min (at room temperature). In the next step, 0.5 mL of the supernatant, 0.5 mL of distilled water and 0.1 mL of $FeCl_3$ (0.1% w/v) were mixed and after 8 min, the absorbance of the solutions was measured. To plot the standard curve, different concentrations (100–1,000 µM) of $FeSO_4{\cdot}7H_2O$ were used. The antioxidant capacity of the tested solutions was calculated using the standard curve which was prepared with a known concentration of Fe^{2+} solution (Eq. 2). The FRAP assay results were reported as µM of Fe^{+2} equivalent per mL of sample.

$$\text{FRAP} = (\Delta A_{sample} / \Delta A_{standard}) \times \text{FRAP value of the standard (µM)} \quad (2)$$

which A_{sample} and $A_{standard}$ are the absorbance of the sample and standard solution, respectively.

2.10 ABTS Assay

The ABTS assay measures the ability of antioxidant compounds to scavenge the ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) generated in an aqueous phase, compared with Trolox as the standard. The total antioxidant activity of kefir and yogurt samples was compared using $ABTS^{\bullet+}$ radical cation decolorization assay (Re et al., 1999). $ABTS^{\cdot+}$ cation radical was generated by the reaction between ABTS (7 mM in water) and potassium persulfate (2.45 mM) (1:1v/v), stored in the dark at ambient temperature for 12-16 h before use. $ABTS^{\cdot+}$ stock solution was diluted with ethanol to obtain a working solution with an absorbance of 0.700 at the wavelength of 734 nm. Then, 0.25 mL of kefir/yogurt extract was added to 3.75 mL of diluted $ABTS^{\cdot+}$ working solution, mixed and the absorbance was

measured at 734 nm after 30 min against a blank. A standard curve was plotted recording the absorbance of different concentrations of Trolox, and the results were reported as mg of TE per 100 mL of sample (Eq. 3).

$$ABTS^{\cdot+} \text{ scavenging effect (mgTE/100ml)} = ((AB–AA)/ AB)\times100 \qquad (3)$$

which AA was the absorbance of control and AB was the absorbance of the sample contained kefir or yogurt extract where AA was the absorbance of control and AB was the absorbance of the sample containing kefir or yogurt extract.

2.11 Statistical analysis

All the experiments were carried out three times. Statistical analyses of data were performed using the statistical software package of SPSS (version 22.0). The results were analyzed by two-way ANOVA to determine the effect of starter culture and storage time on the antioxidant activity. The significance level of 5% was used and data were shown as mean ± standard error of the mean.

3 RESULTS AND DISCUSSION

3.1 Chemical composition

The fat content of ewe, camel, goat and cow milk samples was found 7.14 ± 0.012, 3.58 ± 0.047, 4.03 ± 0.023 and 3.31 ± 0.023 g per 100 mL, respectively. The protein content obtained 6.20 ± 0.105, 3.18 ± 0.055, 3.63 ± 0.075 and 3.45 ± 0.038 g per 100 mL for ewe, camel, goat and cow milk samples, respectively. Total solid content of ewe, camel, goat and cow milk samples, were 19.51% ± 0.80, 11.8%

± 0.65, 12.1% ± 0.48, and 13.5% ± 0.47, respectively. Ewe milk had the highest lactose content (5.05% ± 0.87) followed by cow milk (4.85% ± 0.23), goat milk (4.43% ± 0.39), and camel milk (4.35% ± 0.70).

3.2 pH variations

In dairy-based products, changes in pH play an important role in the quality and organoleptic properties and pH is a key factor that expresses the fermentation activity of starter culture. The growth rate and fermentation capacity of starter microorganisms are extensively varying with the type of milk, nutrients content of milk (protein, lactose, and oligosaccharides) and incubation conditions such as temperature and time (Matar, LeBlanc, Martin, & Perdigón, 2003). The variations of pH values in kefir and yogurt samples during 20 days of storage are shown in Figure 2. We observed a decrease in pH values in all kefir and yogurt samples ($P < 0.05$) depending on the milk source and starter culture. At the beginning phase of fermentation, the pH of kefir and yogurt samples for all milk types were almost similar (between 6.55 and 6.08) and then these values decreased during storage period to achieve the final pH. The final pH was lower in goat and camel and higher in ewe and cow kefir and yogurt. Also, pH values of kefir samples were lower than yogurt samples, independent of milk source, expressing that the traditional kefir starter culture (kefir grain) has conducted the fermentation process more effectively which resulted in decreasing the product pH to the target value. After 24 h (Day 1), the final pH of kefir and yogurt samples were 4.52 ± 0.03-4.63 ± 0.1 and 4.56 ± 0.1-4.68 ± 0.05, respectively; there was no significant difference among the pH values of products prepared from different types of milk ($P > 0.05$). In the kefir samples produced from goat and camel milk, pH decreased to 3.6 5 ± 0.07 and 3.25 ± 0.05, respectively, after 20 days of storage while pH of ewe and cow kefir reached 4.02 ± 0.07 and 4.19 ± 0.03 during the same period ($P < 0.05$). The same results were found by other studies for different types of kefir produced from different starter cultures and pH ranged from 3.64 to 4.05 (Kim et al., 2016). Also, it was reported

by another study that the initial pH of kefir produced from sheep milk was 4.5 and it decreased to 3.70 during the 28-day of the storage period (de Lima et al., 2018). In a study by Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018), the pH of cow kefir was slightly higher than ewe kefir which was similar to our results. Also, the most significant decrease in pH was found for kefir samples produced from grains due to higher metabolic activity compared to commercial starter cultures. It seems that changes in the pH of kefir and yogurt samples during the storage period are due to the difference in buffering potential of kinds of milk and fermentation capacity of different microbial populations used.

Figure 2

3.3 Total phenolic content

The TPC of kefir and yogurt samples showed an increasing trend during the storage period (Figure 3). According to the results, ewe kefir (74.55-80.11 mg of GAE 100 mL^{-1}) showed the highest TPC followed by cow kefir (65-73.15 mg of GAE 100 mL^{-1}), camel kefir (61.2-69.91 mg of GAE 100 mL^{-1}) and goat kefir (58.31-73.5 mg of GAE 100 mL^{-1}) ($P < 0.05$). TPC values of yogurt samples were significantly lower than kefir samples for the same source of milk ($P < 0.05$). Among the yogurt samples, camel yogurt possesses the highest TPC (56.5-68.25 mg of GAE 100 mL^{-1}) followed by ewe (40.32-46.5 mg of GAE 100 mL^{-1}), cow (29.5-35.5 mg of GAE 100 mL^{-1}) and goat (20.03-26.85 mg of GAE 100 mL^{-1}) yogurt ($P < 0.05$). Data obtained for TPC in the present study were almost similar to the results found by Yilmaz-Ersan et al. (2016; 2018) that reported total phenolics as 59.66-66.81 mg of GAE 100 mL^{-1} for goat kefir, 77.74-81.18 mg of GAE 100 mL^{-1} for ewe kefir and 67.41-73.65 mg of GAE 100 mL^{-1} for cow kefir, during the 21-day storage period at 4 ºC. In their study, TPC reached the highest amount at day 14 of storage and then decreased toward the end of storage, but in our work TPC increased throughout the

storage period. Similar results to ours were found by da Silva et al., Sabokbar and Khodaiyan, Bensmira and Jiang who detected an increase in total phenolic compounds during soymilk yogurt production using kefir starter cultures, pomegranate juice and whey based kefir, and peanut based kefir, respectively (Bensmira & Jiang, 2015; da Silva Fernandes et al., 2017; Sabokbar & Khodaiyan, 2016). The decrease or increase in phenolic content could be due to the metabolic activity of microorganisms of starter culture and their capacity to degrade or change the structure of phenolic molecules as it is reported that some yeasts and bacteria could be effective on amount of TPC in fermented dairy products (Apostolidis, Kwon, Shinde, Ghaedian, & Shetty, 2011).

Figure 3

3.4 DPPH radical scavenging potential

DPPH as a stable free radical is soluble in methanol or ethanol, and at the wavelength of 515-520 nm shows characteristic absorption. When this free radical is scavenged by an antioxidant compound by hydrogen donation and the non-radical form DPPH-H is produced, its concentration, color, and absorbance at a given wavelength are reduced (Kulisic, Radonic, Katalinic, & Milos, 2004). According to the results presented in Table 1, ewe and camel milk kefir had the highest radical scavenging potential and inhibitory activity followed by the goat and cow milk kefir during the storage period ($P < 0.05$). We found that the DPPH radical scavenging activity of kefir samples was higher than yogurt samples ($P < 0.05$) and it decreased to the levels lower than the amount observed at day1 in camel, goat and cow yogurt ($P < 0.05$). There were significant differences in the DPPH scavenging potential of kefir and yogurt samples at different storage days ($P < 0.05$). The hydrogen donating capacity of kefir samples

(except for goat kefir) increased during the storage period. The DPPH inhibition values increased considerably in ewe and camel kefir samples during the storage ($P < 0.05$). Similar results were observed by Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018), Bensmira and Jiang (2015), and Sabokbar & Khodaiyan (2016) for kefir with different bases. This notable increase might be due to the hydrolysis of proteins and increased content of organic acids as the result of continuous acidification by starter culture during the storage period (Correia, Nunes, Duarte, Barros, & Delgadillo, 2005). The lowest level of the DPPH scavenging potential was observed in cow yogurt samples ($P < 0.05$), while goat and camel yogurt showed approximately similar hydrogen donating activity at the end of the storage ($P > 0.05$). At day 5, a decrease in antioxidant capacity of goat kefir was detected but after that, it increased significantly ($P < 0.05$). At day 10 of the storage, the antioxidant activity of all yogurt samples decreased. In previous studies, the same results were observed during an extended storage period of fermented dairy products. For instance, Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018) reported a decrease in DPPH value at day 7 for ewe kefir and day 14 for cow kefir. It seems that goat kefir is a good scavenger and hydrogen donor for DPPH radicals and can afford protection against proton free radicals. Also, data from another study showed that DPPH scavenging potential was the highest at day 7 and 14 for cow yogurt and then decreased toward the end of the 28-day storage period as it reached to the level lower than day 1 (A. Shori & Baba, 2013). In the present study, high DPPH inhibition activity after 20-day storage at 4 ºC shows the good metabolic activity of kefir grain microorganisms even at cold temperatures. It is claimed that antioxidative potential of kefir is partly originated from the release of milk peptides by kefir grain microorganisms. It can be suggested that the radical scavenging capacity in kefir is related to proteolysis rate of milk proteins and production of organic acids by the starter culture microflora during fermentation period and storage time. Totally, the diversity of the protein and peptides of the milk and also the microorganisms in the starter culture or kefir grains are determining parameters in antiradical

and antioxidant activity of the products (Suetsuna, Ukeda, & Ochi, 2000). The population and diversity of microorganisms of rural unmodified yogurt or kefir starter cultures differ from modified commercial ones and it seems local cultures possess higher enzymatic and antioxidant activity.

Table 1

3.5 FRAP assay

FRAP assay based on the reduction of a TPTZ (Fe^{2+}) complex to its ferrous form (Fe^{3+}), is one of the common methods to evaluate antioxidant capacity. According to the results (Figure 4), FRAP values for all samples increased toward the end of a 20-day storage period with significant differences between the FRAP data of storage days ($P < 0.05$). In contrast to DPPH free radical scavenging results, no decrease in FRAP values was detected during the storage. It is worthy to note that the pH decrease in the samples was followed by a progressive increase in FRAP values. The FRAP reaction is performed at acidic pH to sustain iron solubility, so a decrease in pH results in a decrease in the ionization potential which facilitates hydrogen transfer and increases the redox potential (Gupta, Caraballo, & Agarwal, 2019). The kefir samples showed higher ferrous reducing capacity compared to yogurt samples ($P < 0.05$). This difference might be attributed to the different microbial populations in kefir grains and yogurt starter culture, their metabolites, and the final pH. The FRAP values of ewe and camel kefir were the highest throughout the storage period ($P < 0.05$). Also, among the yogurt samples ewe and camel yogurt showed the highest FRAP values ($P < 0.05$). The maximum FRAP values were observed after 20 days of storage for all samples, with about a 2-fold increase compared to Day 1 of storage. In a study by Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018), the FRAP values increased during the fermentation period

for ewe and cow kefir produced from kefir grains and a commercial starter culture. The FRAP values of ewe kefir were higher than cow kefir which is similar to our results. In another work, the chelating ability of goat kefir increased during the storage time and the maximum FRAP value was obtained after 21 days of storage (Yilmaz-Ersan et al., 2016), and a similar trend was observed in our study.

The presence of bioactive peptides and functional compounds in milk and diversity of the lactic acid bacteria in the product might explain the high reducing potential of ewe and camel kefir and yogurt. Some fermenting bacteria can produce metabolites that show chelating activity, are able to reduce metal ions and inhibit oxidation reactions (Wang et al., 2017).

Figure 4

3.6 ABTS assay

As shown in Figure 5, the results of ABTS assay for samples were as follows: ewe kefir > camel kefir > ewe yogurt > camel yogurt > cow kefir > goat kefir > goat yogurt > cow yogurt. During the storage period, ABTS scavenging capacity of all samples increased, and kefir samples expressed higher antioxidant activity compared to yogurt samples ($P < 0.05$). It seems that the difference in the antioxidative ability of kefir and yogurt samples during fermentation results from the differences between microorganisms in kefir grains and yogurt starter culture. The same results were obtained by Yilmaz-Ersan, Ozcan, Akpinar-Bayizit, and Sahin (2018). It is reported that high protein content in fermented dairy products resulted in forming oligopeptides, peptones and free amino acids by microbial proteolytic activity and increased antioxidant potential (Tagliazucchi, Martini, & Solieri, 2019). The protein content of ewe, camel, goat and cow milk are approximately 5.41, 3.12, 3.10, and 3.4%, respectively, so it is

obvious that ewe kefir and yogurt had the highest activity in ABTS assay and cow kefir showed higher antioxidant capacity compared to goat kefir (Elbagermi, Alajtal, & Edwards, 2014). Also, there might be a synergistic relation between proteolysis products and phenolic compounds that lead to an increase in the total antioxidant potential of the fermented products (A. B. Shori & Baba, 2014). Thus, variations in antioxidative capacity and ABTS scavenging ability of ewe, camel, goat and cow kefir and yogurt could be attributed to the contents of their protein and amino acid composition, fat and fatty acids, minerals and vitamins (such as vitamin A, C, E), functional compounds such as phenolics and carotenoids, reducing compounds, and type of enzymes (Khan et al., 2019; Ozcan, Sahin, Akpinar - Bayizit, & Yilmaz - Ersan, 2019).

Figure 5

4 CONCLUSIONS

The present study was conducted to compare the antioxidant activity of kefir and yogurt produced from ewe, camel, goat and cow milk. Kefir samples expressed higher antioxidant activity compared to yogurt samples. It demonstrated that the microbial population in kefir grains changed the chemical composition and phenolics of milk and produced metabolites in such a way that led to higher activity in radical scavenging and hydrogen donation of the products. Kefir and yogurt prepared from ewe and camel milk had higher antioxidative potential than samples made from goat and cow milk. The difference between the antioxidant capacity found in kefir and yogurt samples in the present study could be due to multiple parameters like the source of milk, fat, protein, type, and population of microorganisms, variety of enzymes of the kefir grains and starter culture and presence of bio-functional agents with the ability to

donate hydrogen and electron. In future, animal trials, like oral consumption of these fermented products, are needed to investigate the *in-vivo* antioxidative effects of kefir produced from different sources of milk and traditional kefir grains and compare with commercial products.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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506 **Table 1** DPPH radical scavenging activity (mg TE /100mL) of kefir and yogurt samples produced by
507 different types of milk during the storage at 4 °C

Samples	Storage period (day)

	1	5	10	15	20
Ewe kefir	5.18±0.11^{aE}	5.44±0.23^{aD}	5.91±0.09^{aC}	6.3±0.05^{aB}	6.85±0.10^{aA}
Ewe yogurt	4.95±0.08^{bB}	4.97±0.10^{bB}	4.51±0.05^{dC}	4.8±0.09^{cB}	5.03±0.00^{cA}
Camel kefir	5.20±0.15^{eE}	5.63±0.21^{aD}	6.1±0.13^{aC}	6.4±0.18^{aB}	6.93±0.07^{aA}
Camel yogurt	4.90±0.20^{bA}	4.94±0.05^{bA}	4.12±0.00^{eC}	4.36±0.11^{dB}	4.4±0.18^{dB}
Goat kefir	4.48±0.21^{cC}	3.91±0.05^{dD}	5.04±0.10^{bB}	5.25±0.06^{bA}	5.44±0.20^{bA}
Goat yogurt	4.52±0.10^{cA}	4.55±0.15^{cA}	3.9±0.07^{fC}	4.15±0.00^{eB}	4.29±0.25^{dB}
Cow kefir	4.15±0.17^{dE}	4.57±0.04^{cD}	4.83±0.25^{cC}	5.07±0.13^{bB}	5.28±0.21^{bA}
Cow yogurt	3.8±0.10^{eA}	3.88±0.09^{dA}	3.4±0.05^{Gc}	3.58±0.22^{fB}	3.67±0.00^{eB}

$^{a–g}$Different lowercase superscripts in a column express significant difference between means for kefir and yogurt samples ($P < 0.05$).
$^{A–E}$Different uppercase superscripts in a row express significant difference between means during the storage period ($P < 0.05$).