

## Comparison of Some Physical and Chemical Properties of Kefir Obtained from Different Kefir Cultures and Brands of Milk

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Received date:22.06.2022, Accepted date:03.10.2022

### Abstract

In this study, three different kefir cultures were used to ferment five different brands of milk samples. pH, the dry matter, ash amount, carbon dioxide (CO<sub>2</sub>) amount, titratable acidity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capacity, the reducing power, the Fe(II) ions chelating capacity, the total phenolic content (TPC), copper(II) ion reductive antioxidant capacity (CUPRAC) and mineral matter contents were investigated in kefir samples and results were evaluated statistically.

When kefir's CUPRAC, TPC, DPPH free radical scavenging capacity, Mg, Zn, and Na concentrations are evaluated in terms of kefir culture, KC1, KC2, KC3, KC1, KC1, and KC1, had the highest values (p<0.05), respectively. If it is evaluated in terms of different brands of milk, kefir's CUPRAC, TPC, DPPH free radical scavenging capacity and Na concentrations were found highest (p<0.05) which were produced by M5, M2, M1, and M4, respectively.

The data obtained from the experimental studies it was determined that the kefir cultures and milk used had an effect on the quality of the kefir.

**Keywords:** Kefir, kefir culture, milk, antioxidant capacity, mineral

## Farklı Kefir Kültürlerinden ve Süt Markalarından Elde Edilen Kefirin Bazı Fiziksel ve Kimyasal Özelliklerinin Karşılaştırılması

### Öz

Bu çalışmada, beş farklı marka süt örneğini fermente etmek için üç farklı kefir kültürü kullanıldı. pH, kuru madde, kül miktarı, karbondioksit (CO<sub>2</sub>) miktarı, titre edilebilir asitlik, 1,1-difenil-2-pikrilhidrazil (DPPH) serbest radikal temizleme kapasitesi, indirgeme gücü, Fe(II) iyonları şelatlama kapasitesi, toplam fenolik madde (TFM), bakır(II) iyonu indirgeyici antioksidan kapasite (CUPRAC) ve mineral madde içerikleri araştırıldı ve elde edilen sonuçlar istatistiksel olarak değerlendirildi.

Kefirlerin, CUPRAC, TFM, DPPH serbest radikal süpürme kapasitesi, Mg, Zn ve Na konsantrasyonları kefir kültürü açısından değerlendirildiğinde, en yüksek değerlere sırasıyla KC1, KC2, KC3, KC1, KC1 ve KC1 olduğu bulundu (p<0.05). Farklı süt markaları açısından değerlendirildiğinde ise CUPRAC, TFM, DPPH serbest radikal giderme kapasitesi ve Na konsantrasyonlarının en yüksek olduğu kefirlerin sırasıyla M5, M2, M1 ve M4 sütleri kullanılarak üretilen kefirin (p<0.05) olduğu tespit edildi.

DeneySEL çalışmalardan elde edilen veriler, kefir kültürlerinin ve kullanılan farklı marka sütlerin kefirin kalitesine etkisi olduğu belirlendi.

**Anahtar Kelimeler:** Kefir, kefir kültürü, süt, antioksidan kapasite, mineral

### INTRODUCTION

Kefir that originates from the Caucasus Mountains in Russia, is produced from fermented milk drink by using acidic fermentation (Arslan,

2015). This fermented product, which has a slightly sour taste and creamy consistency, is defined as a rich source of probiotic microorganisms (lactic acid bacteria, acetic acid bacteria and yeasts)

accumulated in kefir grain or kefir and used as starter cultures in the preparation of kefir. It is acidic, sour, frothy, slightly alcoholic drink formed by sugar and lactose fermentation in various types of milk (John et al., 2021; Sodanlo and Azizkhani, 2021). It is known that kefir, which is very rich in terms of protein and B vitamins besides potassium and calcium, takes its aroma properties from lactic acid, CO<sub>2</sub> and ethanol, and that these products are formed as a result of fermentation (Beshkova et al., 2003; Perna et al., 2019). Although it shows slight changes depending on the type and content of the milk used in fermentation, its characteristic features are pH about 4.0; alcohol content ranges from 0.5% to 2%; lactic acid; acetic acid; CO<sub>2</sub> and aromatic compounds (Irigoyen et al., 2005; Perna et al., 2019). Kefir that is probiotic drink is typically a homemade fermented product, but it also has the potential to be produced and sold commercially (Farnworth, 2005). It has been reported that regular consumption of kefir improves immunity in the intestinal tract, improves lactose digestion and tolerance, controls blood plasma sugar and blood pressure, as well as has a gastric protective effect (Fahmy and Ismail, 2015; Sodanlo and Azizkhani, 2021). At the same time, it was emphasized that regular kefir consumption is well for ulcers (Fahmy and Ismail, 2015), and that it has gastroprotective effects alongside antimicrobial, anti-allergy, antioxidant, and anti-cancer effects (Bekar et al., 2011; Franco et al., 2013; Ozcan et al., 2019). In addition, it is known that traditionally produced kefir not only improves wound healing, but also lowers cholesterol, reduces allergic effects, and has beneficial effects on the gastrointestinal system. In the past, kefir has been used in traditional medicine as pharmaceuticals without any knowledge of the presence of microorganisms or their therapeutic activities (Arslan, 2015).

There are great number of studies about kefir. However, to our knowledge, there is a lack of studies that compared many physical and chemical properties of kefirs obtained by different kefir cultures and milk samples.

## MATERIAL AND METHODS

### Material

In this study, kefir cultures and milk samples were purchased from different markets. Three different kefir cultures (KC1, KC2, and KC3) were applied to five different brands of ultra-high

temperature (UHT) milks (M1, M2, M3, M4, and M5). Fat content of M1 was 3.5 g, carbohydrate 4.5 g and protein 3.0 g in 100 mL. Fat content of M2 was 3.1 g, carbohydrate 4.7 g and protein 2.8 g in 100 mL. Fat content of M3 was 3.4 g, carbohydrate 4.7 g and protein 3.1 g in 100 mL. Fat content of M4 was 3.3 g, carbohydrate 4.0 g and protein 2.8 g in 100 mL. Fat content of M5 was 3.0 g, carbohydrate 5.0 g and protein 3.0 g in 100 mL.

Each milk sample was fermented separately with 3 different kefir cultures.

### Method

10 g of each of three different kefir cultures and 500 mL of UHT milk from five different brands were taken. Kefir cultures and milks were fermented for 24 h to form kefir. Then, kefir cultures were separated from kefir by using a plastic strainer and stored in refrigerator.

In kefir samples analyzes such as pH, dry matter content, ash content, CO<sub>2</sub> content, titratable acidity, total phenolic content, mineral substance, reducing power, iron(II) ion chelating capacity, copper(II) ion reduction antioxidant capacity, and DPPH free radical scavenging capacity analyses were performed. All analyses were done in triplicate.

### pH

The pH values of the kefir samples were measured using a digital pH meter (Thermo Scientific Orion 3-Star Benchtop).

### Dry Matter

For dry matter analysis, 10 g kefir samples were taken into a beaker and moisture was removed in the oven (Daihan Won-155). Kefir samples were taken from the oven and after cooling in the desiccator samples were weighed (AOAC, 1997).

### Titratable Acidity

For the determination of titratable acidity, 10 mL kefir sample was taken into a beaker and 150 µL of phenolphthalein solution was added then titrated with 0.1 M NaOH. Titratable acidity of kefir samples was calculated as lactic acid % (AOAC, 1997).

### Ash

For ash determination, 20 g kefir samples were taken into a beaker, and the temperature of the furnace (Nüve MF 110) was gradually increased up

to 450 °C. Then it was kept at 450 °C until it turned into white ash (AOAC, 1997).

### Determination of Carbon Dioxide (CO<sub>2</sub>) Amount

The amount of CO<sub>2</sub> was determined titrimetrically. A 10 mL kefir sample was taken from the previously unopened, well-cooled sample, 30 mL of 0.1 M NaOH, 3 mL of 15% BaCl<sub>2</sub> and a few drops of thymol-phthalein indicator were added and mixed well. Titrated with 0.1 M HCl until the pink color disappears (pH 8). For the blank experiment again, 10 mL of kefir sample was taken and the CO<sub>2</sub> was evaporated by boiling for a while. A few drops of thymol-phthalein was added and titrated with 0.1 M HCl until the blue color disappeared (Anonymous, 1976).

The amount of CO<sub>2</sub> was calculated as follows (Equation 1).

$$\text{CO}_2 \text{ amount (mg } 100 \text{ mL}^{-1}) = (a-b) \times 22 \quad (1)$$

a = Amount of 0.1 M NaOH bound by CO<sub>2</sub> = 30 - c

b = Amount of acid consumed in the blank experiment, mL

c = Amount of acid consumed in the sample, mL

### Kefir Samples Extraction for Antioxidant Tests

For antioxidant tests, 10 g of kefir sample was taken and 10 mL of acidified methanol was added, and then mixed in an orbital shaker for 1 h. After the samples were centrifuged, the clear part on the top was taken into a separate test tube. An additional 5 mL of acidified methanol was added to the remaining precipitate and mixed in an orbital shaker for 1 h then centrifuged. The clear portion was combined with the solution previously taken into a separate test tube and kept in the refrigerator until analysis.

### Determination of DPPH Free Radical Scavenging Capacity

Extracts with different concentrations were prepared by taking different amounts of obtained kefir extracts. 250 µL of extracts in different concentrations final volumes were made up to 3 mL with methanol and 1 mL of 1×10<sup>-4</sup> M DPPH solution was added and incubated at room temperature for 30 mins in the dark. At the end of the period, at 517 nm, which is the wavelength DPPH gives maximum absorbance, absorbance was measured with a UV-

Vis spectrophotometer (Shimadzu UV-1800) (Blois, 1958).

DPPH free radical scavenging capacity was calculated according to the formula below (Equation 2).

$$\text{DPPH free radical scavenging capacity \%} = ((A_C - A_K) / A_C) \times 100 \quad (2)$$

A<sub>C</sub>: Absorbance of control at 517 nm

A<sub>K</sub>: Absorbance of kefir at 517 nm

### Total Phenolic Content

The total phenolic content of the extracts of kefir samples obtained with different solvents was determined by modifying the method developed by Singleton and Rossi (1965). After adding 0.5 mL of Folin–Ciocalteu reagent to 0.1 mL of kefir sample, 3 mL of 2% Na<sub>2</sub>CO<sub>3</sub> solution was added 3 mins later, the samples were mixed and incubated for 2 h in the dark. At the end of this period, the absorbance of the samples at a wavelength of 760 nm were measured with a UV-Vis spectrophotometer. Gallic acid was used as standard.

### Determination of Copper(II) Ion Reducing Antioxidant Capacity (CUPRAC)

Determination of copper(II) ion reduction antioxidant capacity was done according to Apak et al. (2004). 1 mL each of 1.0×10<sup>-2</sup> M CuCl<sub>2</sub>, 7.5×10<sup>-3</sup> M neocuproin and 1 M NH<sub>4</sub>Ac (pH 7) was taken. 0.5 mL of kefir extract and 0.5 mL of ultrapure water were added and shaken well. The solutions were incubated for 30 mins at room temperature and then the absorbance values were measured at 450 nm. A concentration-absorbance graph was created for caffeic acid, which is used as a standard in this method.

### Determination of Reducing Power

The reducing force was determined using the Oyaizu (1988) method with some modifications. Kefir extracts and synthetic antioxidants were prepared in the concentration range of 25-500 mg L<sup>-1</sup>. 1 mL is taken from these solutions then 2.5 mL of phosphate buffer (0.2 M and a pH of 6.6), and 2.5 mL of 1% K<sub>3</sub>Fe(CN)<sub>6</sub> solution were added. After the mixture was incubated for 20 mins at 50 °C, 2.5 mL of 10% TCA solution was added. The solution was centrifuged at 2500 rpm for 10 mins. After centrifugation 2.5 mL of supernatants were taken,

Research article/Araştırma makalesi  
DOI:10.29132/ijpas.1134231

mixed with 2.5 mL of ultrapure water and 0.5 mL of 0.1% FeCl<sub>3</sub> solution, and then analyzed by UV-Vis spectrophotometer at 700 nm.

### Determination of Chelating Capacity of Iron(II) Ions

The chelating capacity of iron(II) ions was determined Dinis et al. (1994) method with some modifications. The method is based on the principle of competition between the ferrozine reagent, which is a strong iron chelator, and the metal-binding compounds in the environment to bind Fe<sup>2+</sup> ions. If the chelating power is high, the formation of the red colored Fe<sup>2+</sup>/ferrozine complex is prevented. 3.7 mL of ultrapure water and 100 µL of 2 mM FeCl<sub>2</sub> solution were added to 1 mL of sample. After incubation for 30 mins at room conditions, 200 µL of 5 mM ferrozine solution was added and vortexed. After 10 mins, the absorbance values of the mixtures were measured at 562 nm by UV-Vis spectrophotometer. The control was prepared using 1 mL of ultrapure water instead of the sample. EDTA standard solutions were used at concentrations range of 100-800 mg L<sup>-1</sup>.

### Mineral Content Determination

Approximately 300 mg of kefir sample was taken and 3 mL of concentrated nitric acid and 3 mL of hydrogen peroxide were added and mixed. After waiting for 20 mins, the Teflons were closed; the steps given in Table 1 were applied and resolved in a microwave oven (Microwave system Berghof Speedwave 2). The obtained clear solutions were analyzed by flame atomic absorption spectrophotometer (FAAS). FAAS operating conditions are given in Table 2.

**Table 1.** Microwave oven solubilization steps for kefir samples

Step	1	2	3
Temperature (°C)	145	180	100
Power (%)	75	90	40
Time (min)	5	10	10

### Statistical Analysis

Analysis results were evaluated using the SPSS 22 package program and one way ANOVA test was applied.

## RESULTS AND DISCUSSION

### pH, Dry Matter, Titratable Acidity, Ash and CO<sub>2</sub> Amount of Kefir Samples

The results of pH, dry matter, titratable acidity, ash amount and CO<sub>2</sub> amount of kefir samples are given in Table 3.

The difference between the pH of kefir was found to be statistically significant and it was determined that the pH value of kefir obtained from KC3 culture was lower than other kefir samples, which is thought to be due to microorganism activities. In terms of pH values, Cais-Sokolinska et al. (2008) obtained similar pH values (4.22-5.38). In another study pH of kefir was found 4.59±0.01 that obtained traditional methods (Üstün-Aytekin et al., 2020).

Contrary to pH, statistically no difference was observed between the dry matter amounts of kefir samples obtained from different kefir cultures. In other studies dry matter content of kefir samples found in the range of 11.3%-11.7% and 9.35-13.69% by Iriyogen et al. (2005) and Sady et al. (2007), respectively.

While there was no statistically difference between titratable acidity values of kefir obtained from KC1 and KC2, the titratable acidity of kefir obtained from KC3 was statistically high (p<0.05). Üstün-Aytekin et al. (2020) determined titratable acidity in their kefir samples 0.71 ± 0.00%.

According to obtained results CO<sub>2</sub> amounts of kefir obtained from KC2 (71.3 mg 100 mL<sup>-1</sup>) and kefir obtained from KC1 and kefir obtained from KC3 are similar. Also it was reported that the CO<sub>2</sub> amount increases proportionally the fermentation period of kefir increases (Guzel-Seydim et al., 2005).

In Table 4, no differences were detected in terms of pH, dry matter, titratable acidity, and ash content of kefir samples obtained from different brands of milk. In terms of CO<sub>2</sub> content, kefir obtained from M5 was statistically higher (p<0.05) than kefir obtained from other milks.

A positive, moderate, significant correlation was found between CO<sub>2</sub> content and titratable acidity (r= 0.517\*, p<0.05).

Dry matter and ash amount of kefir samples were calculated as 11.70±0.03% and 0.39±0.01%, respectively by Saygılı (2021).

**Table 2.** FAAS operating conditions for mineral content determination of kefir samples

Element	Acetylene flow rate (L min <sup>-1</sup> )	Air flow rate (L min <sup>-1</sup> )	Wavelength (nm)	Slit range (nm)	Lamp current (mA)
Ca	2.0	17.0	422.7	0.7	20.0
K	2.0	17.0	766.5	0.7	12.0
Mg	2.0	17.0	285.2	0.7	20.0
Na	2.0	17.0	589.0	0.2	12.0
Zn	2.0	17.0	213.9	0.7	20.0

### DPPH Free Radical Scavenging Capacity

This reaction is widely used to test the free radical scavenging capacity or hydrogen donor ability of compounds. In this method, antioxidants reduce the stable radical DPPH to yellow diphenylpicrylhydrazine (Bensmira and Jiang, 2015). Low absorbance indicates a decrease for DPPH radical.

In Table 6, DPPH free radical scavenging capacity of kefir sample obtained from KC3 was found statistically higher ( $p < 0.05$ ) than kefir obtained from KC1 and KC2. Kefirs obtained from KC1 and KC2 showed similar characteristics. The reason for the high value of DPPH free radical removal capacity of kefir obtained by using KC3 may be due to its high microbiological activities.

In Table 7, the DPPH free radical scavenging capacity of kefir obtained from M1 was found to be statistically higher ( $p < 0.05$ ) than the kefir varieties obtained from other milks. Kefir obtained from M3 has the lowest DPPH free radical removal capacity (%).

Marazza et al. (2012), found that the DPPH free radical scavenging capacity in fermented beverages decreased compared to the initial values in unfermented beverages, depending on the fermentation conditions.

Unal (2012), used various kefir samples that taken from the market and extracted. The DPPH free radical scavenging capacity was determined. The DPPH free radical scavenging capacity of the analyzed kefir samples with strawberry and forest fruit was found to be higher than plain kefir.

Yilmaz-Ersan et al. (2016), investigated the effect of fermentation and storage time on DPPH free radical scavenging capacity in kefir obtained from cow's milk. Fermentation time was 0-20 h and storage time was 1-21 days. When fermentation and storage times were compared, DPPH free radical scavenging capacity was found to be the highest at the 8th h ( $7.20 \pm 0.283$ ) and 21st day ( $5.44 \pm 0.198\%$ ),

respectively. The DPPH free radical scavenging capacity of kefir samples is lower than this study.

**Table 3.** Effect of different kefir cultures on pH, dry matter, titratable acidity, ash and CO<sub>2</sub> amount of kefir samples

	Kefir culture			
	KC1	KC2	KC3	SE
pH	5.6b	5.88b	4.64a	0.86
Dry matter (%)	11.1a	12a	11.1a	0.41
Titratable acidity (%)	0.49a	0.42a	0.86b	0.46
Ash amount (%)	1.32a	2.98a	1.96a	0.66
CO <sub>2</sub> amount (mg 100 mL <sup>-1</sup> )	65.2a	71.3ab	87.8b	5.2

a,b: Differences between lines with different letters are significant ( $p < 0.05$ )

SE: Standard error

Goat milk was used for kefir production, kefir samples were stored at 4 °C for 1 and 7 days. It was determined that there was no difference between the DPPH free radical scavenging capacities on the 1st day ( $9.98 \pm 1.42\%$ ) and the 7th day ( $9.98 \pm 6.0\%$ ) (Nurliyani et al., 2015).

In a study DPPH free radical scavenging capacities of plain kefir, black tea added kefir and green tea added kefir samples were determined in the range of 80.88-96.16%. It has been stated that as the storage time increases, the capacity decreases (Karagozlu et al., 2017).

Bensmira and Jiang (2015), compared the DPPH free radical scavenging capacities of peanut milk and kefir samples obtained from peanut milk and determined that the capacity increased as the concentration of the samples increased. They stated that the capacities remained approximately the same when the concentration was more than 20 mg mL<sup>-1</sup>.

Akdan et al. (2020), obtained kefir by using buffalo milk and cow, sheep and goat milk, which

**Table 4.** Effect of different brands of milk on pH, dry matter, titratable acidity, ash amount and CO<sub>2</sub> amount of kefir samples

		Different brands of milk					
		M1	M2	M3	M4	M5	SE
Kefir	pH	5.5a	5.4a	5.2a	5.4a	5.4a	0.11
	Dry matter (%)	11.2a	11.5a	11.9a	11.3a	11.2a	0.53
	Titratable acidity (%)	56a	53a	67a	57.6a	63a	5.9
	Ash content (%)	3.7a	1.6a	1.6a	1.7a	1.7a	0.8
	CO <sub>2</sub> content (mg 100 mL <sup>-1</sup> )	66.5a	76ab	71.6ab	67.7a	92b	6.7

a,b: Differences between lines with different letters are significant ( $p < 0.05$ )  
 SE: Standard error

they mixed with buffalo milk in certain proportions. It was reported that the lowest DPPH radical scavenging activity was obtained by using only buffalo milk, and the highest by using a mixture of buffalo and cow's milk, with the first day storage of kefir samples.

#### Total Phenolic Content

The total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu reagent. The TPC was calculated as gallic acid equivalent (GAE). Linear regression equation and correlation coefficient obtained from gallic acid calibration curve were  $y = 0.0007x + 0.0635$  and  $R^2 = 0.9977$ , respectively.

In Table 6, it was found that the TPC amount of kefir obtained from KC2 was statistically higher ( $p < 0.05$ ) than kefir obtained from other kefir cultures, and kefir obtained from KC1 and KC3 was similar.

In Table 7, it was determined that kefir obtained from M2 was the statistically highest ( $p < 0.05$ ) in terms of TPC amount, while kefir obtained from M3 was the lowest.

It has been determined that the amount of TPC may increase depending on the fermentation time of lactic acid bacteria or other microorganisms (Vuong, et al., 2006).

In a study, the amount of TPC was determined in kefir samples obtained from cow's milk at different fermentation and different storage times. The TPC was found to be  $170.54 \pm 0.198$  mg g<sup>-1</sup> when the fermentation time was 0 h, and it was determined that there was a decrease in the amount of TPC as the fermentation time increased. However, when the storage time was examined, an increase was observed in the amount of TPC as the

time increased. While the amount of TPC on the 1st day was  $59.66 \pm 0.085$  mg g<sup>-1</sup>, it was determined as  $66.81 \pm 0.156$  mg g<sup>-1</sup> on the 21st day, which was the last storage period (Yilmaz-Ersan et al., 2016).

Nurliyani et al. (2015) determined the TPC amount of kefir samples obtained from goat milk was  $4.60 \pm 0.75$  mg 100 mL<sup>-1</sup> on the 1st day, and  $5.94 \pm 1.03$  mg 100 mL<sup>-1</sup> on the 7th day. The results obtained are similar to these results.

The TPC of peanut milk and kefir samples obtained from peanut milk was compared and it was stated that the TPC amount of kefir obtained from peanut milk was considerably higher than peanut milk (Bensmira and Jiang, 2015).

**Table 5.** Correlation analysis results of the properties of kefir samples

	pH	Dry matter	Titratable acidity	CO <sub>2</sub> content
pH	1	0.34	-0.97**	-0.558*
Dry matter	0.34	1	-0.298	-0.122
Titratable acidity	-0.97**	-0.298	1	0.517*
CO <sub>2</sub> content	-0.558*	-0.122	0.517*	1

\*\* $p < 0.01$ , \* $p < 0.05$

#### Copper(II) Ion Reducing Antioxidant Capacity (CUPRAC)

Caffeic acid was used as a standard for the determination of copper(II) ion reducing antioxidant capacity of kefir extracts. The linear regression equation and correlation coefficient obtained from caffeic acid calibration curve were  $y = 0.0362x + 0.1957$  and  $R^2 = 0.9969$ , respectively.

In Table 6, it was found that the CUPRAC value of kefir obtained from KC1 was statistically

high ( $p < 0.05$ ), and kefir obtained from KC2 and KC3 were similar.

As seen in Table 7, it was determined that the CUPRAC of kefir obtained from M5 was high, and kefir samples obtained from M2 and M3 were the lowest. On the other hand, kefir obtained from M1 and M4 indicated similar results.

### Reducing Power

In the reducing power method, the presence of antioxidants in the sample allows  $Fe^{3+}$  to be reduced to  $Fe^{2+}$  by giving electrons. The amount of  $Fe^{2+}$  complex is determined by measuring the blue color formed at 700 nm. A rising absorbance value

indicates an increased ability to reduce (Ebrahimzadeh et al., 2009).

In this study, kefir samples of different concentrations ( $25 \text{ mg L}^{-1}$ - $500 \text{ mg L}^{-1}$ ) were prepared and the reducing powers of these samples were compared. In addition, the reducing power of kefir samples was compared with synthetic antioxidants such as ascorbic acid and butyl hydroxyl toluene.

According to the data obtained, it was found that, as the concentration of kefir samples increased, the reducing power also increased, but the reducing power of kefir samples were lower than the synthetic antioxidants ascorbic acid and butylated hydroxyl toluene.

**Table 6.** Effect of kefir culture on DPPH free radical scavenging capacity, TPC, CUPRAC, Ca, K, Mg, Na and Zn contents of kefir samples

	Kefir culture			
	KC1	KC2	KC3	SE
DPPH free radical scavenging capacity (%)	13.7a	14.2a	18.8b	0.3
TPC (mg GAE $\text{kg}^{-1}$ )	32.8a	44.8b	31.3a	0.7
CUPRAC (mg CAE $\text{kg}^{-1}$ )	72.9b	67.6a	69.4a	1.0
Ca (mg $\text{kg}^{-1}$ )	60a	56a	61a	3
K (mg $\text{kg}^{-1}$ )	1155a	1138a	1113a	35
Mg (mg $\text{kg}^{-1}$ )	43b	32a	29a	2
Na (mg $\text{kg}^{-1}$ )	458b	416a	409a	12
Zn (mg $\text{kg}^{-1}$ )	4.0b	3.2a	2.9a	0.2

a,b:Differences between lines marked with different letters are significant ( $p < 0.05$ )  
SE: Standard error

**Table 7.** Effect of different brands of milk on DPPH free radical scavenging capacity, TPC, CUPRAC, Ca, K, Mg, Na and Zn contents of kefir samples

	Different brands of milk					
	M1	M2	M3	M4	M5	SE
DPPH free radical scavenging capacity (%)	24.9e	16.4d	9.8a	14.6c	12.5b	0.4
TPC (mg GAE $\text{kg}^{-1}$ )	37.0c	50.7d	28.4a	32.9b	32.5b	0.9
CUPRAC (mg CAE $\text{kg}^{-1}$ )	70.6b	65.1a	61.8a	72.1b	80.3c	1.3
Ca	55a	55a	60a	63a	61a	4
K	1110ab	1095a	1157ab	1245b	1070a	45
Mg	34a	33a	36a	34a	35a	2
Na	437b	381a	427ab	505c	388a	15
Zn	3.0a	3.5ab	3.4ab	3.8b	3.4ab	0.2

a,b,c,d,e: Differences between lines marked with different letters are significant ( $p < 0.05$ )  
SE: Standard error

In a study, peanut milk and kefir samples obtained from it were used and it was stated that the reducing power of kefir samples were higher than the reducing power of milk samples and this was due to fermentation (Bensmira and Jiang, 2015).

Sabokbar and Khodaiyan (2016) investigated the effects of fermentation, temperature and the amount of kefir grains on antioxidant capacity in the beverage they prepared using pomegranate juice and kefir. It was stated that beverages fermented at 25 °C with 8% (w/v) kefir grains had the highest reducing power and the absorbance value at 700 nm was  $0.951 \pm 0.032$ , and the reducing power increased as the fermentation temperature increased.

### Iron(II) Ions Chelating Capacity

Metal chelating capacities of kefir extracts were compared with EDTA. It was determined that the iron(II) ion chelating capacity of EDTA was higher than the iron(II) ion chelating capacity of kefir samples. Among the kefir samples, it was determined that the iron(II) ion chelating capacity of kefir was the highest obtained from KC2 and M2 than the other samples.

Liu et al. (2005), determined that there was no change in the iron(II) ion chelating capacity of kefir obtained with milk and soy milk.

### Mineral Matter Content

Linear regression equation and correlation coefficient were calculated as  $y = 0.0209x + 0.0014$   $R^2 = 0.9969$  for Ca,  $y = 0.0161x - 0.0013$   $R^2 = 1$  for K,  $y = 0.4491x + 0.011$   $R^2 = 0.9981$  for Mg,  $y = 0.1426x + 0.0061$   $R^2 = 0.9968$  for Na and  $y = 0.2624x + 0.0176$   $R^2 = 0.9958$  for Zn.

There was no difference between kefir cultures in terms of Ca and K content. In terms of Zn, Mg and Na content, kefir obtained from KC1 was highest ( $p < 0.05$ ) (Table 6).

Calcium plays a role in the regulation of muscle contraction, blood coagulation and cell membrane permeability. Magnesium is an important macromineral. It is known that calcium and magnesium are associated with the regulation of the heart muscle (Turker et al., 2013). When Table 7 was examined, obtained from different milk brands were found to be similar in terms of Ca and Mg. Generally dairy products contain significant amounts of Ca.

Zinc, which plays a role in metabolic reactions, is an important source of dairy products (Wang et

al., 2018). It was found that kefir obtained from M1 had the lowest concentration in terms of Zn content.

It is known that the K mineral found in milk plays a role in the regulation of osmotic pressure and transmission of nerve impulses. Na is required for the regulation of osmotic pressure and acid-base balance in the human body (Turker et al., 2013).

In terms of K and Na content, kefir obtained from M4 was found to be the highest while kefir obtained from milk M5 was found to be the lowest for K and M2 for Na.

Turker et al. (2013), carried out mineral substance analysis in kefir and milk samples of cow and goat. Ca ( $1674.5 \pm 67.8$  mg L<sup>-1</sup>) and Mg ( $111.3 \pm 5.2$  mg L<sup>-1</sup>) concentrations of kefir samples obtained from cow milk is higher than cow milk Ca and Mg concentrations. However, Na concentration ( $444.6 \pm 9.3$  mg L<sup>-1</sup>) is higher in milk than kefir. There is no statistical difference between zinc concentrations. Similarly, Ca ( $1793.0 \pm 7.9$  mg L<sup>-1</sup>), Mg ( $175.8 \pm 2.1$  mg L<sup>-1</sup>) and Na ( $395.4 \pm 3.6$  mg L<sup>-1</sup>) concentrations of kefir samples obtained from goat milk are higher than goat milk Ca, Mg and Na concentrations. There is no statistical difference between zinc concentrations. While the K and Na values in the study are similar to this study, the Ca and Mg concentrations are quite high compared to this study.

### CONCLUSION

In present study three different kefir cultures and five different brands of milk used to obtain kefir samples. The results of kefir samples were statistically evaluated in terms of kefir cultures and different brands of milk. It has been determined that kefir culture and different brands of milk have an effect on the antioxidant properties of kefir such as DPPH, TPC, and CUPRAC, that is, antioxidant properties change as kefir culture and milk change. In addition, it was found that kefir culture and different brand milk had no effect on the Ca content of kefir, which is rich in minerals.

Despite the high consumption of milk and dairy products, the inadequacy of kefir consumption is due to the lack of awareness that kefir is a good natural food source for health. Kefir is especially important for health due to its high antioxidant activity and mineral substance content. Considering to this study results, it was concluded that the generalization of kefir consumption would contribute positively to public health.

Research article/Araştırma makalesi  
DOI:10.29132/ijpas.1134231

## ACKNOWLEDGEMENT

This study was supported by The Scientific Research Projects Coordination Unit of Munzur University. Project Number: YLMUB016-16.

## CONFLICT OF INTEREST

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

## RESEARCH AND PUBLICATION ETHICS STATEMENT

The authors declare that this study complies with research and publication ethics.

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