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Korrespondenzadresse:
nilgunoncul@mu.edu.tr

[1]) Muğla Sıtkı Koçman University, Fethiye Faculty of Health Sciences, Department of Nutrition and Dietetics, Karaçulha 48300 Fethiye/Muğla, Turkey; [2]) Niğde Ömer Halisdemir University, Faculty of Engineering, Department of Food Engineering, 51240 Niğde, Turkey

Microbiological, antimicrobial and antioxidant characteristics of commercial Turkish kefirs

Mikrobiologische, antimikrobielle und antioxidative Eigenschaften kommerzieller türkischer Kefirs

Nilgün Öncül[1]), Ayşe Özbey[2]), Hakan Erinç[2]), Zeliha Yildirim[2])

Summary

In this study, the microbiological properties, physicochemical characteristics, antimicrobial and antioxidant activities of Turkish commercial kefir products were investigated. The enumeration of Lactococci, aerobic lactobacilli, anaerobic lactobacilli, acetic acid bacteria (AAB) and yeast were performed by spread plate technique and counted as 7.35, 6.62, 6.85, 6.24 and 2.35 log CFU/g, respectively. Antimicrobial activity of kefir was evaluated using the disk diffusion method. Ten microorganisms, including seven Gram-positive strains and three Gram-negative strains were tested. While *Listeria monocytogenes, L. ivanovii* and *Escherichia coli* were the sensitive bacteria for all kefir samples, *L. plantarum, Staphylococcus aureus, Bacillus cereus* and *E. coli* O157:H7 are the resistant bacteria. The means of pH, total phenolic content, Trolox Equivalent Antioxidant Capacity (TEAC) and lipid peroxidation were 4.03, 4.39 mg GAE/100 mL, 0.15 µmol Trolox/mL and 7.23%, respectively. From the beneficial health properties point of view, kefir appears to be a promising food.

Keywords: antimicrobial, antioxidant, commercial, kefir, pathogens

Introduction

Kefir is a fermented milk product which has gained considerable interest with its nutritional values and functional properties (Guzel-Seydim et al., 2005; Kim Jeong et al., 2018). Kefir is a sour, acidic, self-carbonated and low alcoholic product with a unique taste (Iraporda et al., 2017; Manthani et al., 2018). Kefir is produced from kefir grains that surrounded by a polysaccharide matrix called ‚kefiran' (Weschenfelder et al., 2018). Kefir grains are composed of lactic acid bacteria, acetic acid bacteria and yeast, in which a complex microbiota live symbiotically (Guzel-Seydim et al., 2005; Iraporda et al., 2017; Nale et al., 2018). These microorganisms are responsible for lactic, acetic and alcoholic fermentations which are important for the characteristic properties of kefir (Iraporda et al., 2017).

Despite that kefir is a traditional dairy beverage originated from Caucasus region, it is one of the most popular fermented milk products currently in lots of the countries include European, United States, etc (de Lima et al., 2018; Shi Chen et al., 2018). Its consumption has increased in recent years due to its promoting effect on human health (Gul et al., 2018). It has been recommended for its functional properties such as anticarcinogenic, antibacterial, antiallergenic, antiasthmatic, antidiabetic immune-modulation and cholesterol-lowering effects. These effects may be related to the presence of a complex microbiota and their metabolites which are produced during the fermentation process (Iraporda et al., 2017; Temiz and Dağyıldız, 2017; Weschenfelder et al., 2018; Karaçalı et al., 2018).

Kefir is obtained by traditional or commercial methods which based on adding kefir grains or starter culture to milk for fermentation (Iraporda et al., 2017; Gul et al., 2018). Traditional kefir production is started with pasteurization of milk (85–90°C, 20 minutes) and continued cooling down it at 20–25°C. Kefir grains (2–10%) are inoculated into pasteurized milk and incubated at 20–25°C for 18–24 hours. After incubation, the kefir grains are removed by sieving and kefir are kept at 4ºC until consumption. The sieved kefir grains are left to dry at room temperature before refrigeration until next production. It can be also stored by freezing (Terzi 2007; Yıldız-Akgül et al., 2018). The basic steps of industrial manufacture of kefir are homogenization of milk, heat treatment (90–95°C, 5-10 minutes), cooling (18–24°C), the addition of starter culture (2–8%) and fermentation (18-24 hours). After fermentation, the fermented product is separated by filtration and bottled before maturation (3–10°C, 24 hours) at 4°C (Terzi, 2007; Kim et al., 2018).

There have been several scientific researches on the health benefits of kefir products that have been attributed to the composition of microorganisms and metabolic products. It is well known that the presence of them is affected by a number of factors especially production methods. Kefir and its promising effect on health have been studied for many years; however, the researches which investigate the leading roles of this effect such as microbiological flora, antioxidant capacity and antimicrobial properties are limited. The chemical-free living conditions, spending less time cooking and routinizing fast-food consumption habits are affected the consumer preference. Consumers have been tending to healthy, natural, less processed and chemical-free foods. So, some tend, which have functional properties besides their high nutritional values, have gained popularity. This tends caused that the products produced traditionally and consumed locally are evaluated as an industrial product. Consumers are tent to prefer industrial products because of modern life conditions. So, these products have become widespread between countries and even continents. Kefir, as a traditional dairy product, started to present as a commercial product and this caused to question of the benefit of kefir by some consumers. This work has been planned to provide an answer to commercial kefir products for the quality and safety of commercial kefir products in terms of public health. Besides the conventional kefir, industrial products such as light and fruity kefirs are offered as an option to the today's consumer. It is thought that the analysis of some microbiological and physicochemical properties of fruity and plain kefir and question kefir products belonging to different brands for meeting different consumer demands can as a source of information. Therefore, the present study was conducted to assess the microbial properties, antimicrobial and antioxidant activities of the industrially produced Turkish kefir products (plain and fruity).

Material and Methods

Collection of Samples

Two types of kefir products, plain and fruity, sold in different retail markets in Tokat province were investigated in this study. The kefir products were obtained from 3 different manufacturers, a total of 21 samples were evaluated in 3 different time spans in July and August. Kefir products that have passed no more than one week from the date of production were collected as samples. All the samples were transferred to the laboratory in a thermobox within original packages. The package material of the samples is plastic bottle which are made with high density polyethylene (HDPE). The plain and fruity kefir samples were purchased in their originals package of 1 L and 250 mL, respectively. Nine plain kefirs (1, 2 and 3) and 12 fruity kefirs (4, 5, 6 and 7) were analyzed. The product's details were presented in Table 1. All the kefir samples were produced from cow milk. All experiments were carried out with two replicates and two parallels.

TABLE 1: *Details about the kefir products according to the labels.*

NUTRITION VALUES	SAMPLES						
	1 (250 mL)	2 (100 g)	3 (100 g)	4 (250 mL)	5 (100 g)	6 (250 mL)	7 (100 g)
Energy (kcal)	113.1	58	52.4	113.1	83.25	135.3	83.25
Carbohydrate (g)	10	5	3.2	10	8.85	16.5	8.85
Protein (g)	6.8	4	2.7	6.8	3.7	6.75	3.7
Fat (g)	5.1	3	3.2	5.1	3.25	4.7	3.25

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Test cultures

Pure cultures containing *Escherichia coli* ATCC 3509, *E. coli* O157:H7 ATCC 35150, *Salmonella* Typhimurium RSKK 95091, *Listeria monocytogenes* ATCC 7644, *Listeria ivanovii* RSKK 93036, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* RSKK 623, *Lactobacillus plantarum* DSM 2601, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* RSKK 863 were used as indicator bacteria for antimicrobial activity analysis. These bacterial cultures were obtained from the Refik Saydam National Type Culture Collection (RSKK) and Ankara University. Stock cultures were kept at –80°C in Brain Heart Infusion Broth (BHIB) with 20% glycerol. The stock cultures were activated twice in BHIB at 37±2°C for 18–24 hours.

Microbiological properties

The microbiological analyses were conducted to determine the microbiological properties of kefir samples. A weight of 10 g of each sample was added into the 90 mL peptone water (0.1%) and homogenized by a stomacher for 90 s. Afterward; serial dilutions were carried out for the enumeration of Lactococci, aerobic lactobacilli, anaerobic lactobacilli, acetic acid bacteria (AAB) and yeast.

The counts of Lactococci were made on M17 agar (pH 7.2±0.2). Plates were incubated at 30°C for 24–48 hours in an anaerobic jar (Terzaghi and Sandine, 1975). Aerobic lactobacilli were enumerated on de Man, Rogosa and Sharpe (MRS) agar. Plates were incubated at 30°C for 24 hrs. For lactobacilli growth under anaerobic conditions, the anaerobic jar system was used at the same incubation conditions (Lee et al., 2006). Acetic acid bacteria were enumerated on acetic acid bacteria (AAB) medium (3% yeast extract, 0.02% BCG, 2% ethanol, 2.5% mannitol and 1.5% agar) at 30°C for 1–5 days (Carr and Passmore, 1979). Yeasts were grown on Potato Dextrose Agar (PDA) at 25°C for 5 days (Mossel et al., 1995). After counting, means and standard deviations were calculated and the results were expressed as log CFU/g.

Antimicrobial activity

Antimicrobial activities of kefir samples were evaluated using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI M02, 2015; CLSI M45, 2016). Paper disks were kept in kefirs for 30 min. Then the paper disks with kefir were applied to the agar surface previously inoculated with organism suspension (10^8 CFU/mL) (CLSI M07-A8, 2009). After incubation for 24 hours at the conditions required by each indicator strain, the inhibition zones were measured.

Physico-chemical properties

The pH, total phenolic content and the antioxidant capacities of the samples were determined. The pH values were measured by a calibrated pH meter (WTW Inolab pH Level 1, Germany) (AOAC, 1995). Total phenolic content (gallic acid equivalent) and antioxidant activity of kefirs were analyzed spectrophotometrically. The total phenolic content was determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). The antioxidant capacities of kefirs were evaluated using the Trolox Equivalent Antioxidant Capacity by ABTS (TEAC) The TEAC values of the samples were tested according to the method described by (Re et al., 1999).

Lipid peroxidation assay was done with little modification (Sirirat and Jelena, 2010). A volume of 0.1 mL of sample and 0.4 mL of water was mixed with 0.5 mL of egg yolk solution (20% v/v). Then this solution was vortexed well with 0.07 mL of FeSO4 (10 mM) and incubated for 30 min at room temperature. After adding 1.5 mL of thiobarbituric acid solution 0.8% (w/v) (thiobarbituric acid in 1.1% sodium dodecyl sulfate) samples were mixed well and heated for 60 min (95°C). After samples were cooled, 5 mL of n-butanol was added. The samples were centrifuged for 5 min at 6000 rpm. The supernatant was used and absorbance of each sample was measured at 532 nm. BHT at same conditions was used as a control. The antioxidant activity was given as an inhibition percentage and was calculated as:

$$\%\,\text{Inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analyses

All statistical analyses were performed with the SPSS statistical package program (IBM SPSS Statistics Version 22; USA). All experiments werecarried out with two replicates and two parallels. The significant difference between the means was established by ANOVA variance analysis and Duncan Tests. Independent-Samples T-Test was applied to compare the mean of the sample's groups. The significance levels of $P < 0.05$ were used for statistical differences.

Results and Discussions

Microbiological properties

The mean values of Lactococci, aerobic Lactobacilli, anaerobic Lactobacilli, AAB and yeast for kefir samples were 7.35, 6.62, 6.85, 6.24 and 2.35 log CFU/g, respectively (Table 2). There were no statistically significant differences between the means of plain and fruity kefirs in tested microorganisms ($p>0.05$) except Lactococci.

The Lactococci levels detected by Guzel-Seydim (2005) and Grønnevik et al., (2011) for Turkish and Norwegian kefir samples were slightly higher than the ones described in this study. The result of Lactococci was lower than Cetinkaya and Mus (2012). These authors examined 50 kefir samples purchased from different retail markets in Bursa province and found the count of 8.25 log CFU/mL. On the other hand, lower or higher values of Lactococci loads than that of the current work have been reported by other some authors in kefir. Irigoyen et al., (2005) and Witthuhn et al., (2005) obtained counts of 10^6 and 3.5×10^6 CFU/mL for Lactococci after 30 days of storage. By the way, Yildiz-Akgül et al. (2018) detected higher counts in the range of 9.5–10.2 log CFU/mL for Lactococci. Perna et al. (2019) worked on donkey milk kefir and they stated higher counts of Lactococci (9.13 log CFU/mL), Lactobacilli (10.07 log CFU/mL) and yeast (6.96 log CFU/mL) after 15 day of storage.

In this investigation, the mean count of aerobic and anaerobic Lactobacilli were found be 6.62 and 6.85 log CFU/mL, respectively. These mean counts of Lactobacilli in plain and fruity kefir samples were lower than the result of Dinç (2008) who noted the mean counts of Lactobacilli as 8.36 and 8.32 log CFU/mL for plain and fruity kefirs sold in Ankara. The counts of Lactobacilli in our study were 1 log unit lower than those observed by Cetinkaya and Mus (2012). These findings of Lactobacilli are consistent with those reported by Irigoyen et al. (2005) and Witthuhn et al. (2005). In another research, the count of Lactobacilli ranged between 7.477–8.505 log CFU/g in kefir samples which produced from goat, ewe and cow milk (Öner et al., 2009). These results were higher than the mean value of Lactobacilli (6.74 log CFU/

TABLE 2: *Microbial properties of kefir samples (log CFU/g).*

Kefir Samples		Lactococci	Aerobic Lactobacilli	Anaerobic Lactobacilli	Acetic Acid Bacteria	Yeast
Plain	1	7.40±0.13^{b*}	6.39±0.15^{a}	6.44±0.18^{a}	6.33±0.12^{bc}	<1±0.00^{a}
	2	7.78±0.11^{c}	7.03±0.11^{c}	7.21±0.08^{cd}	5.92±0.29^{a}	5.15±0.19^{d}
	3	8.16±0.15^{d}	6.19±0.17^{a}	6.78±0.36^{b}	6.27±0.23^{bc}	2.34±0.05^{b}
Fruity	4	6.38±0.12^{a}	6.24±0.10^{a}	6.37±0.06^{a}	6.26±0.15^{bc}	<1±0.00^{a}
	5	7.61±0.16^{bc}	6.64±0.14^{b}	7.00±0.11^{bc}	6.11±0.06^{ab}	4.47±0.03^{c}
	6	6.59±0.08^{a}	6.66±0.10^{b}	6.79±0.08^{b}	6.57±0.08^{c}	<1±0.00^{a}
	7	7.55±0.11^{b}	7.19±0.01^{c}	7.35±0.02^{d}	6.20±0.15^{ab}	4.50±0.04^{c}
Plain	Mean**	7.78±0.35^{a}	6.54±0.40^{a}	6.81±0.39^{a}	6.17±0.27^{a}	2.49±2.23^{a}
Fruity	Mean	7.03±0.59^{b}	6.68±0.36^{a}	6.88±0.38^{a}	6.29±0.21^{a}	2.24±2.34^{a}

*Means with the standard deviations, n=12 different lowercase letters indicate differences between rows ($p<0.05$).
**Means with the standard deviations of plain or fruity kefir samples. Different lowercase letters indicate differences between rows ($p<0.05$).

mL) which obtained in this study. Atalar (2019) mentioned that Lactobacilli and Lactococci counts were both present at 9.0–9.5 log CFU/mL in freshly fermented kefir samples. After 20 days of storage at 4°C, the counts were decrease to 7–8 log CFU/mL. In this view, the results of this study are quite similar. The count of Lactobacillus, Lactococcus and Leuconostoc remained higher than 7.8 log CFU/g during the storage period at 4°C in soymilk kefir samples that were slightly higher (da Silva Fernandes et al. (2017).

In the present survey, the average values of AAB were 6.17 and 6.29 log CFU/g for plain and fruity kefir samples. These results were similar to those obtained by Irigoyen et al. (2005) and Witthuhn et al. (2005). They found AAB counts of 10^6 CFU/mL and 2.2×10^6. The counts of AAB in Brazilian kefir samples were higher than our result. The findings for AAB were noted as 7.72 and 7.20 log CFU/mL by Magalhães Pereira et al., (2011) and Leite et al. (2013). Nevertheless, the result (6.24 log CFU/g) of AAB was higher than the count observed by Loretan Mostert and Viljoen (2003) in South Africa kefir samples.

The yeasts levels of the plain and fruity kefir samples were detected as 2.49 and 2.24 log CFU/g, respectively. However, for yeasts, the Turkish Food Codex Communique on Fermented Milk (TGK, 2009/25) and Codex Standard for Fermented Milks (Codex Stan 243-2003) list 10^4 CFU/g as minimum yeast content for kefir. The count of yeasts for samples 1, 4 and 6 was below the undetectable level. The sample 3 also wasn't meet the required level. On the other hand, the amount found in kefir sample 2, 5 and 7 analyzed in this study were higher and meet the required level. Yeast amounts in kefir vary and reported values range from 10^3 to 10^8 (Irigoyen et al., 2005; Guzel-Seydim 2005; Witthuhn et al., 2005; Fontán et al., 2006; Öner et al., 2009; Magalhães et al., 2011; Leite et al., 2013; Temiz and Kezer, 2014). At the same time, results were considerably lower than the counts observed by Cetinkaya and Mus (2012) which found $7.7\text{x}10^4$ CFU/mL and Dinç (2008) which were present at levels of 4.05 and 3.23 log CFU/mL of yeast for plain and fruity kefir. Atalar (2019) reported that the yeast counts was 2 log CFU/mL in the fresh kefir samples and it was slightly increased 2.7 log CFU/mL after 20 days of storage. The yeast counts of kefir samples 1, 3, 4 and 6 were found below the limit value of 4 log CFU/mL, which is specified by the Fermented Milks Codex. In another study, it was reported that the number of yeast in goat milk kefir produced with a commercial kefir yeast decreased during storage (up to 30 days) and the yeast count was 4.38 log CFU/mL as a result of analysis performed on the last day of storage(O'Brien et al., 2016).

Antimicrobial activity

The antimicrobial activity of the kefir samples was tested on a total of ten microorganisms, including seven Gram-positive bacteria strains and three Gram-negative bacteria strains. Table 3 shows the inhibitory zone diameter for tar-

TABLE 3: *Antimicrobial activity of kefir samples (Inhibition zones mm*).*

Bacteria	SAMPLES						
	PLAIN			FRUITY			
	1	2	3	4	5	6	7
Lactobacillus plantarum DSM 2601	-	-	-	-	-	-	-
Enterococcus faecalis ATCC 29212	-	7	8	-	8	-	9
Enterococcus faecium RSKK 623	-	7	-	-	7	-	8
Listeria monocytogenes ATCC 7644	7	8	8	8	9	9	12
Listeria ivanovii RSKK 93036	7	8	7	7	8	8	10
Staphylococcus aureus ATCC 25923	-	-	-	-	-	-	-
Bacillus cereus RSKK 863	-	-	-	-	-	-	-
Escherichia coli ATCC 3509	7	7	8	8	8	9	9
Escherichia coli O157:H7 ATCC 35150	-	-	-	-	-	-	-
Salmonella Typhimurium RSKK 95091	-	7	-	-	7	-	8

*Diameter of disc was 5 mm -. no inhibition.

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get microorganisms. It was observed that *L. monocytogenes, L. ivanovii* and *E. coli* are the sensitive bacteria for all kefir samples tested and *L. plantarum, S. aureus, B. cereus* and *E. coli* O157:H7 are the resistant bacteria. Kefir samples 2, 5 and 7 had a slightly more inhibitory effect than the others. These samples had inhibition zones for six of the tested bacteria. The sample 7 had the biggest antimicrobial zone diameters among the kefir samples. The higher inhibitory effect of this kefir sample is thought to be due to the fact that it is fruity kefir and its pH is low.

Microbiological and physicochemical properties of kefir samples may influence their antimicrobial activity against microorganisms. pH values, phenolic compounds, antioxidants, antagonistic action of various microorganisms present in kefir are responsible for the inhibition (de Lima et al., 2018; Weschenfelder et al., 2018). The microbial populations of kefir grains are capable of producing a wide range of antimicrobial compounds, including organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins). These compounds interact each other to enhance or antagonize their antimicrobial effects (Chifiriuc, Cioaca and Lazar, 2011; İsmaiel et al., 2011; Leite et al., 2013; Kim et al. 2016; de Lima et al., 2018; Weschenfelder et al., 2018). As it is known, antimicrobial activity can also express with different methods. Moretti et al. (2019) established a minimum inhibitory content (MIC) value for *S.* Enteritidis and *E. coli* of 20% v/v of samples. In another study, Cetik Yildiz et al. (2019) were stated the inhibitory effect of kefir samples with MIC method as 2.42, 7.9 and 4.55 mg/mL for *S. aureus, P. aeruginosa* and *E. coli* respectively. Assessment of the antibacterial activity of goat milk kefir on *E. coli* ATCC 8739 and *S.* Typhimurium ATCC 14028 were measured with using a well diffusion method. The inhibitory zones were 2.98 and 2.34 mm for *E. coli* ATCC 8739 and *S.* Typhimurium ATCC 14028 (Said et al., 2019).

Ismaiel et al. (2011) were conducted to elucidate the antimicrobial effect of kefir samples towards some bacteria and fungi, including *S. aureus, B. cereus* and *E. coli.* These results showed a better antimicrobial effect which in turn 14, 13 and 11 mm, if compared with the results of this study. Another research was performed to determine the inhibitory effect of kefir samples against 13 different microorganisms. For target strain, the diameter of inhibition zones ranged from 11.11 mm to 20.50 mm (Garrote et al., 2000). Similar results were reported by other researchers. These studies were done with several microorganisms comprising *S. aureus, B. cereus, E. coli, E. faecalis, L. monocytogenes* and *S.* Typhimurium. Chifiriuc et al. (2011) were reported the antimicrobial activity of kefir against Gram-positive and Gram-negative bacteria. Ulusoy et al., (2007) were obtained the antimicrobial effect against tested bacteria with the diameter zones from 17.9 mm to 21.2 mm. Rodrigues et al., (2005) were investigated the antimicrobial activity of kefir samples against pathogenic strains. The researchers were indicated that the inhibition zones were varied between 24.9 to 30.2 mm. However, Sirirat and Jelana (2010) reported that the tested kefir samples did not have any inhibitory effect against *S. aureus, B. subtilis* and *Pseudomonas fluorescents* they were only effective against *E. coli.* Kim et al. (2016) were noted the antibacterial effect of kefir samples towards eight pathogen and spoilage bacteria. Although *B. cereus* and *P. aeruginosa* were responded strongly to the inhibitory effect, no inhibition zone was observed for *E. faecalis.* As similar, all tested kefir samples in this research did not show any effect against *S. aureus, B. cereus, E. coli* O157:H7 and *L. plantarum.*

The reasons for the different results obtained in antimicrobial activity studies with kefir are thought to be due to the use of commercial kefir culture or kefir granules in its production, the differences in the microbial composition of kefir culture or kefir granules and the different target species or strains of target bacteria used in the antimicrobial activity test. Degree of fermentation also affects the antibacterial activity of kefir. The microbial composition in the grains and kefir starter cultures is variable and largely dependent on source, the fermentation process conditions such as fermentation time, temperature, degree of agitation, type of milk, grain/milk inoculum ratio and microorganism distribution, among others (Ahmed et al., 2013; Ajam and Koossari, 2020; Leite et al., 2013; Rattray and O'Connel, 2011; Sindi et al., 2020).

Physicochemical properties

Table 4 presents the values of the main physicochemical parameters in the kefir samples.The means of pH, total phenolic content, TEAC and lipid peroxidation were 4.03, 4.39 mg GAE/100 mL, 0.15 µmol Trolox/mL and 7.23%, respectively. No significant differences in the pH and lipid peroxidation values of the plain and fruity kefir samples were found ($p>0.05$). However, the total phenolic content and TEAC values of the plain and fruity kefir samples were significantly different ($p<0.05$).

With respect to Table 4, the pH values of kefir samples ranged from 3.94 to 4.13. Cetinkaya and Mus (2012)

TABLE 4: *Physico-chemical properties of kefir samples.*

Samples		pH		Total Phenolic Content mg GAE/100mL sample		Trolox Equivalent Antioxidant Capacity (TEAC) µmol trolox/mL sample		Lipid Peroxidation %	
PLAIN	1	4.13±0.06^{c*}	4.04±0.09^{a}	3.87±0.18^{a}	3.96±0.25^{a}	0.14±0.02^{a}	0.14±0.01^{a}	9.51±0.26^{e}	6.89±2.18^{a}
	2	3.94±0.02^{a}		3.93±0.36^{a}		0.14±0.02^{a}		4.51±0.15^{b}	
	3	4.08±0.03^{bc}		4.09±0.21^{a}		0.15±0.02^{ab}		6.66±0.26^{cd}	
FRUITY	4	4.05±0.04^{b}	4.01±0.07^{a}	4.25±0.10^{a}	4.70±0.32^{b}	0.14±0.01^{a}	0.16±0.01^{b}	7.07±0.22^{d}	7.48±3.46^{a}
	5	3.97±0.03^{a}		4.99±0.16^{b}		0.17±0.01^{ab}		3.68±0.08^{a}	
	6	4.11±0.02^{bc}		4.75±027^{b}		0.17±0.01^{ab}		12.78±0.39^{f}	
	7	3.94±0.04^{a}		4.82±0.11^{b}		0.17±0.00^{b}		6.38±0.23^{c}	

*Means with the standard deviations, n=12 Different lowercase letters indicate differences between rows ($p<0.05$).

reported the pH value 4.3 for commercial kefir collected from different retail markets in Bursa province (Turkey). Nateghi et al. (2016) investigated the physicochemical properties of kefir obtained from a local store in Iran and reported the pH value of 3.69. Slightly higher pH values were detected by Dinç (2008) who found 4.26 and 4.13 for plain and fruity kefir samples sold in Ankara (Turkey). da Silva Fernandes et al. (2017) reported that pH values of soymilk kefir were between 4.53–4.72 during the 41 days of storage at 4°C.

The total phenolic content of the samples varied between 3.87 and 4.99 mg GAE/100 mL. Yilmaz-Ersan et al. (2016) indicated the phenolic content 66.81 GAE mg/g for goat milk kefir samples after 21 days of storage. Total phenolic compound was increased during the storage period (up to 21 days) for cow (67.41–64.18 mg GAE/100 mL) and ewe milk kefir (77.74–80.89 mg GAE/100 mL) and even the beginning day these samples had quite higher values than the samples of this study (Yilmaz-Ersan et al. (2018). Folin-Ciocalteu reducing capacity of donkey milk kefir (4.96 mg GAE/100 g) was higher even the first day of storage at 4°C (Perna et al., 2019). Total phenolic content was tested in soymilk kefir and the result was lower even the last day of storage (3.93 (mg GAE/g) (da Silva Fernandes et al. (2017). Kefir samples which are included fruits are higher values compared to plain ones for both total phenolic content and TEAC. Fruity kefirs produced with puree, sauce or powder of red fruits such as strawberry, raspberry and blackberry are rich in phenolic content (Bermúdez-Soto and Tomás-Barberán, 2004). Donkey milk kefir produced with honey has higher antioxidant capacity than plain one as similar result of this study (Perna et al., 2019). The antioxidant capacity were measured with ABTS and FRAP and found 14.98 mg TE/100g and 4.24 mg TE/100g for plain kefir and 16.61 mg TE/100g and 7.75 mg TE/100g for kefir with honey. There is no validated method that can reflect all the antioxidant property of the food. So, many different assays are used to determine capacity. The antioxidant capacity of the samples can be measured by several methods such as DPPH, TEAC, FRAP, etc. (Huang et al., 2005). TEAC values were found from 0.14 to 0.16 µmol Trolox/mL in this study. It is important to use at least two complimentary methods for evaluating the antioxidant capacity in food. Lipid peroxidation was also performed and changed from 3.68 to 12.78%. As expected, fruity kefirs had higher antioxidant capacities than plain ones. The antioxidant activity of kefirs samples was measured by DPPH free radical scavenging activity and chelating effect and obtained as 28.67±0.31% and 11.97±0.22%, respectively (Shi et al., 2018). DPPH (5.44 mg Trolox equivalent (TE)/100 mL), ABTS (11.14 mg Trolox equivalent (TE)/100 mL) and FRAP (7.13 mg Trolox equivalent (TE)/100 mL) methods were displayed by Yilmaz-Ersan et al. (2016) for investigating the antioxidant activity of kefirs in the end of the storage period. DPPH scavenging activities were found 10.84% for kefirs after fermentation (Karaçalı et al., 2018). Liu et al., (2005) was investigated that the inhibition rate of cow-milk kefir and goat-milk kefir upon linoleic acid peroxidation was 88.6% and 76.0%, respectively. Yilmaz-Ersan et al. (2018) was presented antioxidant capacity of cow and ewe milk kefir made using kefir grains and starter culture by DPPH, ABTS and FRAP. There were no significant differences among ewe kefir produced with grain or starter culture in terms of DPPH and ABTS. However, cow milk kefir had significant differences. The values of DPPH, ABTS and FRAP were 5.41, 14.08 and 6.40 mg TE/100 mL for kefir produced with grain and 3.40, 15.05 and 6.04 mg TE/100 mL for kefir produced with starter culture.

Overall, the microbiological properties, antimicrobial and antioxidant activity of the samples were varied in this investigation. This variety of kefir depends on many factors such as kind of milk and milk composition, type of grains and technological conditions. These differences can be associated with the method of production, origin of grain, type of starter cultures, fermentation process, time, temperature, degree of agitation, type of milk, grain/milk inoculum ratio, microorganism distribution, storage temperature and time, addition of ingredients (fruit puree, sauce, powder, etc.) and standardization of milk. These factors can influence microbiological and chemical characteristics of the final kefir product (Sady, Domagała et al., 2007; Dinç, 2008; Leite et al., 2013).

Conclusion

The research reported in this study was to gain insight into some probiotic properties of Turkish commercial kefir products. As a conclusion, the results show that commercial kefir products have potential as functional food in terms of their viable count of specific microflora, antimicrobial properties and antioxidant capacity.

For the consumer, the microbiological and chemical composition of industrial kefir products indicates a complex probiotic effect. In terms of these beneficial health properties, kefir is valuable food and assures further studies.

Authors' contributions

N.Ö carried out the experiment and wrote the manuscript with support from Z.Y. A.Ö. developed the theory and performed the computations. A.Ö helped supervise the project. H.E verified the analytical methods and contributed to sample preparation. Z.Y conceived of the presented idea and supervised the project. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Address of corresponding author:
Nilgün Öncül, Ph.D.
Muğla Sıtkı Koçman University
Fethiye Faculty of Health Sciences,
Department of Nutrition and Dietetics, Karaçulha
48300 Fethiye/Muğla
Turkey
nilgunoncul@mu.edu.tr
nilgunoncul@hotmail.com