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**Research Paper / Araştırma Makalesi**

# Use of Cow and Buffalo Milk with Different Fat Contents for Production of Kefir Drinks with Kefir Grain and Starter Culture: Their Protein and Tyrosine Contents during Storage

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## ABSTRACT

In this study, cow and buffalo milk with different fat contents were used for the production of kefir drinks with kefir grain or kefir culture, and the protein and tyrosine contents of kefir drinks were determined during 21 days of storage at 4°C. The protein content of samples decreased during storage (P<0.05). Protein contents of kefir samples ranged from 3.44 to 4.80% (P<0.05) at the beginning and from 3.31 to 4.71% at the last day of storage. Tyrosine content of kefir samples increased for the first 14 days of storage, and it decreased at the end of storage except for the kefir produced with cow milk (P<0.05). Tyrosine contents of samples produced with starter culture were higher than those with kefir grains, and the use of cow milk had a significant effect on the tyrosine content of kefir samples (P<0.05). At the end of storage, the highest tyrosine content (15.80 µg/g) was determined in kefir from cow milk with a 0.5% fat content and starter culture, and the lowest (8.04 µg/g) was determined in kefir from buffalo milk with a 3% fat content and kefir grain (P<0.05).

**Keywords:** Cow milk, Buffalo milk, Kefir, Protein, Tyrosine

## Farklı Yağ İçeriklerine Sahip İnek ve Manda Sütünün Kefir Danesi ve Starter Kültürle Kefir İçeceği Üretiminde Kullanılması: Depolama Süresince Protein ve Tirozin İçerikleri

## ÖZ

Bu çalışmada, farklı yağ oranlarında inek ve manda sütüyle kefir danesi veya kefir kültürü kullanılarak üretilen kefirlerin, 4°C'de 21 günlük depolama süresince protein ve tirozin içeriği üzerindeki etkisi incelenmiştir. Örneklerin protein içeriği depolama boyunca düşüş göstermiştir (P<0.05). Örneklerin protein içerikleri depolama başlangıcında %3.44-4.80 arasında (P<0.05), depolamanın son gününde ise %3.31-4.71 arasında değiştiği saptanmıştır. Örneklerin tirozin içerikleri depolamanın ilk 14 günü boyunca artmış, inek sütü ile üretilen kefirler hariç depolama sonunda azalmıştır (P<0.05). Starter kültürle üretilen örneklerin tirozin değerlerinin daha yüksek olduğu ve tirozin içeriğine inek sütünün önemli derecede etkisinin olduğu belirlenmiştir (P<0.05). Depolama sonunda en yüksek tirozin değeri, 15.80 µg/g ile %0.5 yağlı sütle starter kültür kullanılarak üretilen inek sütü kefirlerinde; en düşük değer ise, 8.04 µg/g ile %3 yağlı sütle dane kullanılarak üretilen manda sütü kefirlerinde tespit edilmiştir (P<0.05).

**Anahtar Kelimeler:** İnek sütü, Manda sütü, Kefir, Protein, Tirozin

## INTRODUCTION

Fermented dairy products have an important place in milk technology as well as in human nutrition and protection of health. Kefir, which is one of the most consumed fermented milk products in the world, takes a second place after yogurt [1]. Having acidic features and a mild alcoholic taste, kefir has an important place among fermented dairy products [2]. It is believed to be originated from the mountainous regions of the Caucasus [3, 4]. Kefir has a different feature compared to other fermented milk products. It is traditionally produced from irregularly shaped, gelatinous grains that resemble small, yellowish-colored cauliflowers [4-6]. These grains consist of a highly complex matrix, called kefiran, which is comprised of a water-soluble polysaccharide, together with a complex microflora, such as lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc and Streptococcus*), yeasts and sometimes acetic acid bacteria, as well as. Microflora is a part of a thin polysaccharide matrix resulting from the microbial metabolism of lactose [7]. The matrix contains microorganisms together with casein (30-34%), polysaccharides (45-60%), and fat (3-4%) [8]. The number and variety of microorganisms that make up kefir in traditional kefir production (by using grains) may vary according to the source, the method and the substrates used, therefore, the end product features are not standardized [9, 10]. Therefore, the use of cultures prepared to standardize kefir production is becoming increasingly widespread. The microbial structure of the grains is highly suitable for isolating pure cultures. The lactobacillus constitutes the largest part (65-80%) of the microbial population. The remaining part consists of *Lactococcus* and yeast [11].

Peptides and amino acids are produced by starter cultures as a result of proteolytic activity in fermented dairy products [12]. Tyrosine is the one of these amino acids. Therefore, one of the methods used to determine the level of proteolysis is the tyrosine value in fermented dairy products. The total amount of amino acids released as a result of proteolysis is expressed as the tyrosine value. Tyrosine is a bioactive peptide that has large spectrum of functional activity including the analgesic activity, antioxidant activity, antidiarrhoeal properties, and antidepressant activity [13-15].

Kefir can be produced from the milks of cow, goat, sheep, camel, buffalo with all kinds of fat rates, as well as herbal milk such as soy milk, rice milk and coconut milk [3, 10, 16]. In this study, it is aimed to determine the changes in tyrosine and protein contents of kefir, which is produced by using kefir grain or kefir culture with cow and buffalo milk in different fat ratios during 21-day storage at 4°C.

## MATERIALS and METHODS

### Materials

Kefir grains used in the study are from Ankara University, Faculty of Agriculture, Milk Technology Department of Education-Research and Application, and starter cultures are provided from Chr. Hansen firm (Chr. Hansen Inc., Denmark). Characteristics of the used kefir culture; FD-DVS eXact® KEFIR 2 in mesophilic/thermophilic kefir culture, and in its composition, it contains the following microorganism mixture: *Lactococcus lactis* spp. *cremoris*, *Lactococcus lactis* spp. *lactis, Lactococcus lactis* spp. *lactis* var. *Diacetylactis*, *Leuconostoc* spp., *Debaryomyces hansenii*, *Streptococcus thermophilus*. Before use, kefir grains were activated at 22-24°C by using UHT-treated milk. Cow and buffalo milk used in production were obtained from a local dairy farm (Afyonkarahisar, Turkey).

### Methods

Kefirs used in the research were produced from cow and buffalo milk in different fat rates (fat-free, semi-fat and full-fat) by using kefir grain and starter culture. Production of kefir was made with a revised version of the method presented by Kabak and Dobson [17] (Figure 1). The produced kefir was stored for 21 days at 4±1°C in 250 mL amber glass bottles. Chemical analyses were conducted on the 1$^{st}$, 7$^{th}$, 14$^{th}$ and 21$^{st}$ days of storage.

The dry matter, pH, titration acidity, fat, protein and lactose contents of raw milk and the samples to be processed into kefir, which were used as raw materials, were determined according to AOAC methods [18]. Kjeldahl method was used to determine the protein content of kefir samples. For this purpose; 5 mL samples were taken into Kjeldahl tubes and catalytic tablets made of potassium sulfate and mercuric oxide were placed on the surface, and incinerated by the addition of sulfuric acid. Afterwards, distillation and titration processes were performed respectively and total nitrogen value was calculated. The protein content was calculated by multiplying the determined total nitrogen value with a protein factor (6.38) [19].

The determination of tyrosine content of kefir samples were performed using HPLC method (Shimadzu Prominence Kyoto, Japan). 25 g of kefir samples were collected for the analysis and 25 mL of 0.1 M HCl was added. Then the homogenized mixture was centrifuged at 4000 rpm for 4 minutes at 4°C. After removing the supernatant, 100 µL 2 N NaOH, 150 µL saturated sodium bicarbonate, and 1 µL dansyl chloride was added. The mixture was incubated at 40°C for 45 minutes, and kept at room temperature for 10 minutes. Then, 50 µL of 25% $NH_3$ was added. 5 mL ammonium acetate: acetonitrile was added to the mixture, which was kept at room temperature for another 30 minutes. It was injected into the HPLC system after filtering on 0.45 µm [20, 21].

*HPLC operating conditions:* Detector: DAD (SPD-M20A), Mobile Phase: A: 0.1M Ammonium acetate, B: Acetonitrile, Column: ACE5 C-18 (250x4.6 mm, 5 µm), Column Temperature: 40°C, Flow rate: 1 mL/min 46, Injection volume: 50 µL and the results were evaluated at 254 nm. The recovery values were 80%.

## Statistical Analyses

Experiments were conducted in triplicates with two-parallels for each. Statistical analyses were performed by using the SPSS 16.0 statistical package program [22]. The data were evaluated via the variance analysis technique (ANOVA) in chance-based blocks trial plan. The Duncan test was used to determine the level of differences in the groups demonstrating significant differences.

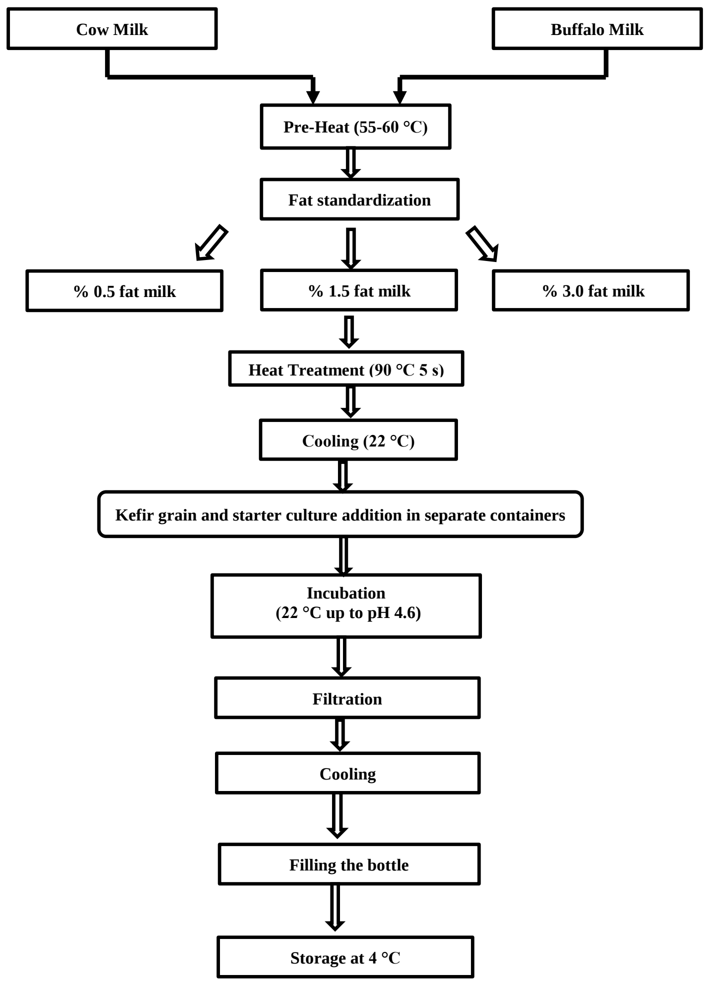


Figure 1. Flow diagram of kefir production

## RESULTS and DISCUSSION

Some chemical characteristics of cow and buffalo milk processed in kefir are shown in Table 1. On the first day of storage, protein contents of kefir samples ranged from 3.44 to 4.80% ($P<0.05$) and on the last day of storage, it ranged from 3.31 to 4.71% ($P<0.05$) (Table 2). Halle [23] determined that protein contents of kefir samples were between 3.10 and 4.72%. When the protein content of standardized cow and buffalo milk used in production was examined (Table 1), it was determined that this change was between 3.49-3.55% in cow's milk and 4.57-4.82% in buffalo milk. The variability in the protein contents of kefir samples might be due to the differences in the composition of the milk used in their production (Figure 2).

Table 1. The chemical composition of cow and buffalo milk used in production of kefir

| Properties | Cow milk | | | Buffalo milk | | |
|---|---|---|---|---|---|---|
| | Fat content (%) | | | Fat content (%) | | |
| | 0.5 | 1.5 | 3 | 0.5 | 1.5 | 3 |
| Dry matter (%) | 9.21 | 9.86 | 11.08 | 12.05 | 13.02 | 14.28 |
| pH | 6.46 | 6.44 | 6.43 | 6.63 | 6.60 | 6.62 |
| Acidity (°SH) | 7.02 | 7.03 | 7.05 | 8.57 | 8.59 | 8.60 |
| Fat (%) | 0.50 | 1.50 | 3.00 | 0.50 | 1.50 | 3.00 |
| Protein (%) | 3.55 | 3.51 | 3.49 | 4.82 | 4.69 | 4.57 |
| Lactose (%) | 3.80 | 3.75 | 3.68 | 4.76 | 4.71 | 4.64 |

From the perspective of different fat ratios, a general decrease was determined in all of the three fat levels in the protein content of all kefir samples during storage (P>0.05) (Figure 3). The decrease in the protein content of kefir samples during storage could be explained by the proteolytic effect due to microbial activities in kefir [24]. In current study, it was determined that the production method (kefir grain or starter culture) had an insignificant difference in the protein content of kefir samples during storage (Figure 4).

Table 2. Changes in protein contents of kefir during storage (%)

| Milk Type | Fat Content of Milk (%) | Production Method | Storage time (day) | | | |
|---|---|---|---|---|---|---|
| | | | 1* | 7 | 14 | 21 |
| Cow | 0.5 | Grain | 3.52DEa | 3.45EFGb | 3.39GHc | 3.35GHc |
| Cow | 1.5 | Grain | 3.47EFa | 3.41FGb | 3.36Hbc | 3.31Hc |
| Cow | 3.0 | Grain | 3.44Fa | 3.39Gab | 3.36Hbc | 3.32Hc |
| Cow | 0.5 | Starter Culture | 3.53Da | 3.48Eab | 3.44Fbc | 3.41Fc |
| Cow | 1.5 | Starter Culture | 3.49DEFa | 3.46EFab | 3.42FGbc | 3.37FGc |
| Cow | 3.0 | Starter Culture | 3.47EFa | 3.44EFGab | 3.41FGab | 3.38FGb |
| Buffalo | 0.5 | Grain | 4.77Aa | 4.70Bb | 4.66Bbc | 4.62Bc |
| Buffalo | 1.5 | Grain | 4.65Ba | 4.60Cab | 4.56Db | 4.54CDb |
| Buffalo | 3.0 | Grain | 4.52Ca | 4.47Dab | 4.43Ebc | 4.39Ec |
| Buffalo | 0.5 | Starter Culture | 4.80Aa | 4.77Aab | 4.73Abc | 4.71Ac |
| Buffalo | 1.5 | Starter Culture | 4.68Ba | 4.64BCab | 4.61Cbc | 4.57Cc |
| Buffalo | 3.0 | Starter Culture | 4.55C | 4.53D | 4.52D | 4.50D |

*a-c (→): Values with similar letters within a row for each analysis differ significantly (P<0.05),
A-G (↓) Values with similar capital letters within a column for each analysis differ significantly.

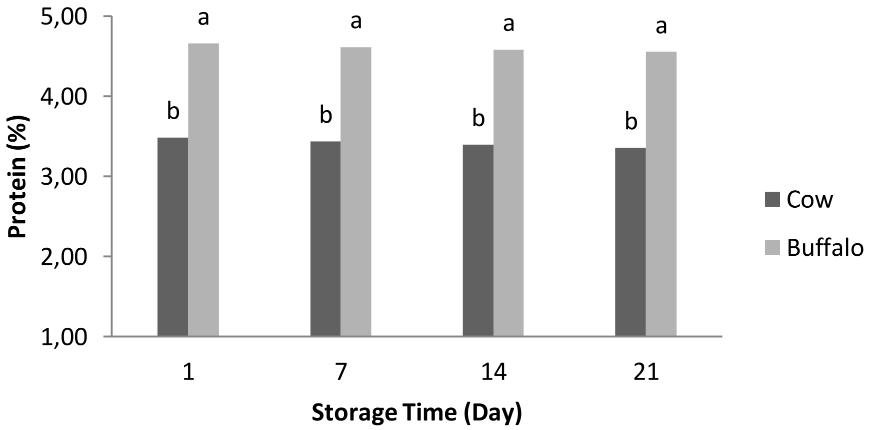


Figure 2. Effect of milk type on protein contents of kefir samples during storage

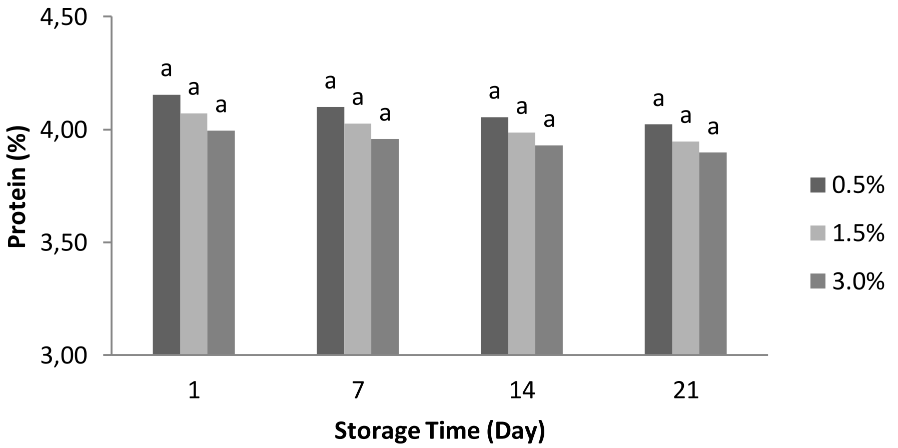


Figure 3. Effect of milk fat content on protein contents of kefir samples during storage

Ersoy and Uysal [25] determined a decrease in protein contents of kefir samples separately produced with both grain and starter cultures during storage. In current study, the protein content of kefir produced by kefir grain was 2.35%; and it was determined as 2.27% in the kefir produced with starter culture. However, in a study on kefirs produced from cow and soy milk with kefir grain, which was conducted by Liu [26], protein content was 6.6% in kefir obtained from cow milk and 9.6% in kefir obtained from soy milk.

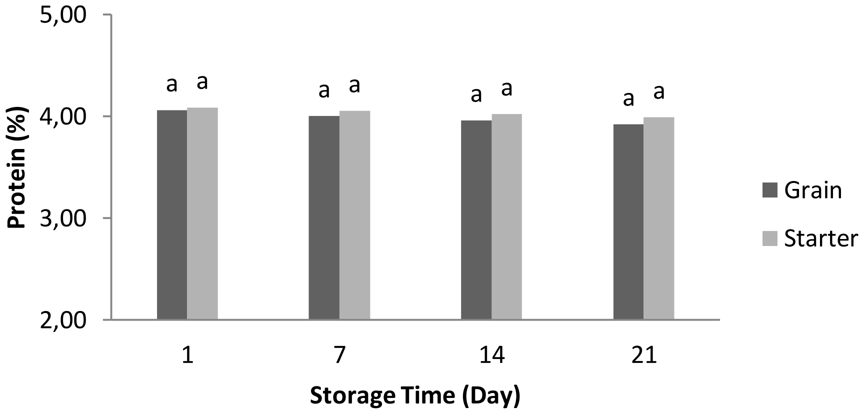


Figure 4. Effect of production method on protein content of kefir samples during storage

Tyrosine is one of the nitrogenous compounds generating from the decomposition reactions of proteins as a result of the proteolytic activities of the starter cultures in the fermented dairy products [10]. Tyrosine content is used to determine the level of kefir-like fermented milk proteolysis. At the beginning of storage, tyrosine content of samples ranged from 4.86 to 14.60 g/g ($P<0.05$) (Table 3). When tyrosine contents of kefir samples were compared in general, the tyrosine values of samples produced with starter culture were higher than those of kefir with grain. The tyrosine values of the samples generally increased during the 14 days of storage ($P<0.05$) (Figure 5). However, on the 21st day of storage, a partial decrease in kefir produced with cow milk, and an increase in kefir produced with buffalo milk were determined (Figure 6). Ender [27] reported that tyrosine values of kefir samples increased during 30 days of storage. Similarly, Ersoy and Uysal [25] determined that tyrosine content in kefir samples increased during the 9-day storage period.

Table 3. Tyrosine contents of kefir samples during storage (µg/g)*

| Milk Type | Fat Content of Milk (%) | Production Method | Storage time (day) | | | |
|---|---|---|---|---|---|---|
| | | | 1* | 7 | 14 | 21 |
| Cow | 0.5 | Grain | 11.68Dc | 12.68Db | 13.28Da | 13.05Dab |
| Cow | 1.5 | Grain | 11.05Ec | 11.92Eb | 12.56Ea | 12.36Eab |
| Cow | 3.0 | Grain | 10.52Fc | 11.44Fb | 12.32Fa | 12.04Ea |
| Cow | 0.5 | Starter Culture | 14.60Ac | 16.08Ab | 16.60Aa | 15.80Ab |
| Cow | 1.5 | Starter Culture | 13.14Bc | 14.48Bb | 15.24Ba | 15.03Ba |
| Cow | 3.0 | Starter Culture | 12.48Cd | 13.80Cc | 14.68Ca | 14.32Cb |
| Buffalo | 0.5 | Grain | 5.94Jc | 7.35Jb | 8.61Ha | 8.83Ha |
| Buffalo | 1.5 | Grain | 5.28Kd | 6.63Kc | 7.95Ib | 8.43Ia |
| Buffalo | 3.0 | Grain | 4.86Ld | 6.36Kc | 7.56Jb | 8.04Ja |
| Buffalo | 0.5 | Starter Culture | 7.62Gd | 8.76Gc | 9.78Gb | 10.14Fa |
| Buffalo | 1.5 | Starter Culture | 7.26Hd | 8.34Hc | 9.24Hb | 9.63Ga |
| Buffalo | 3.0 | Starter Culture | 6.93Id | 8.01Ic | 8.85Hb | 9.36Ga |

*a-d (→): Values with similar letters within a row for each analysis differ significantly ($P<0.05$),
A-L (↓)Values with similar capital letters within a column for each analysis differ significantly.

The effect of fat content and milk type on the tyrosine content of kefir samples during storage is shown in Figures 5 and 6, respectively. Cow milk had a significant effect on the tyrosine content of samples ($P<0.05$). At the end of storage, it was determined that the highest tyrosine value was 15.80 µg/g and 0.5% in the cow-milk kefir produced from full-fat milk with starter culture, while the lowest value was 8.04 µg/g and 3% in the buffalo-milk kefir produced from full-fat milk with grains. Similarly, Gul [28] determined a lower tyrosine value in samples produced with buffalo milk, when he/she examined the tyrosine content of kefir produced by using cow and buffalo milk.

Tyrosine content of samples produced with starter culture was higher than samples produced with kefir grains (Figure 7) ($P<0.05$). Gul [28] found a higher tyrosine value in kefirs produced by starter culture and grain, and relatively higher values in samples produced with starter culture. On the other hand, in the stored kefir samples, Ersoy and Uysal [25] found that tyrosine contents of kefir samples produced from grains were higher than those produced with culture.

Kılıç [29] stated that the proteolytic activity value was higher in kefir samples produced from grain than kefir samples produced from starter culture. Differences could be generated from the use of different starter cultures and grains. Proteolytic activity has importance in terms of acid-forming abilities of starter cultures and also in sensory properties of kefir [30].

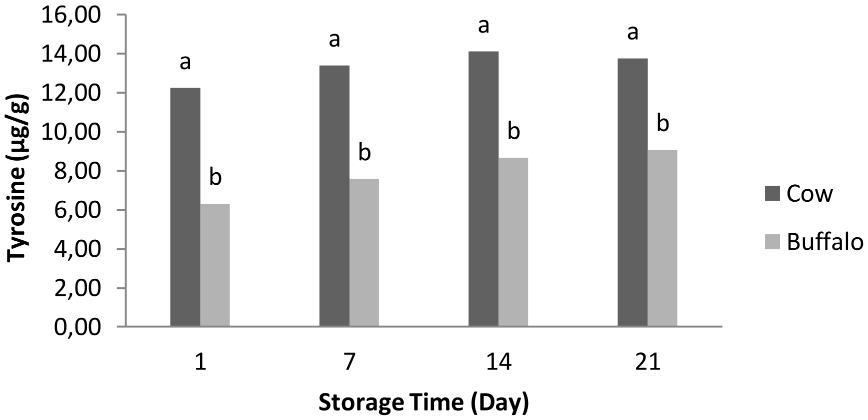


Figure 5. Effect of milk type on tyrosine content of kefir samples during storage

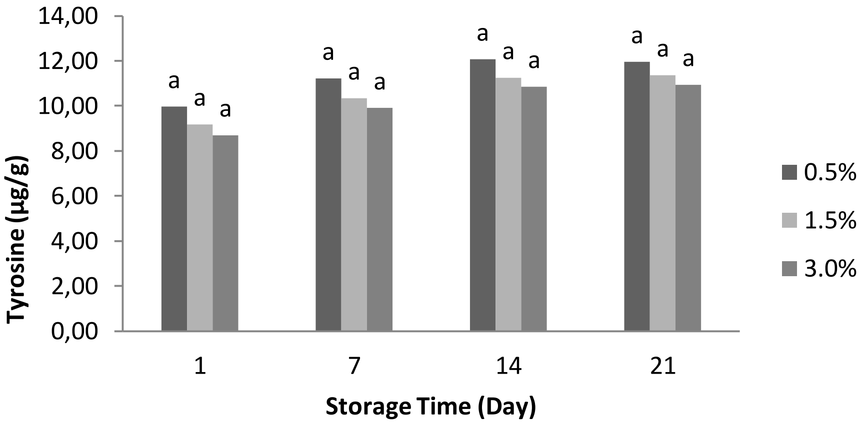


Figure 6. Effect of milk fat content on tyrosine contents of kefir samples during storage

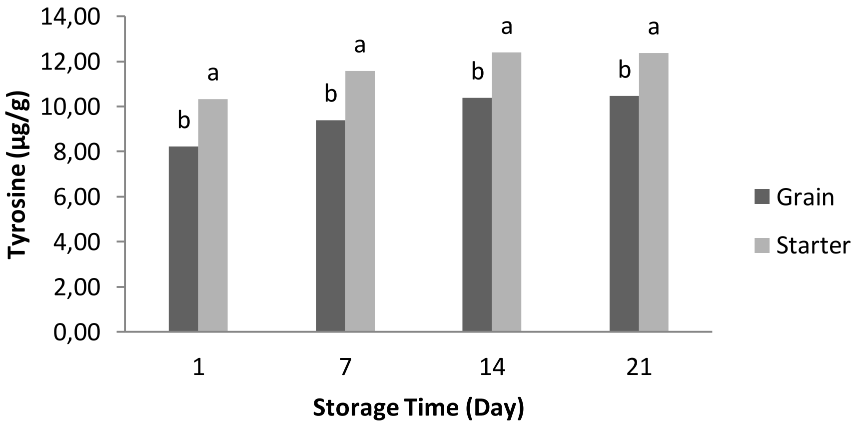


Figure 7. Effect of production method on tyrosine contents of kefir samples during storage

## CONCLUSIONS

Buffalo milk is a raw material alternative to cow milk for the production of kefir, and chemical characteristics of kefir produced with buffalo milk in this study revealed that it can easily be an alternative to kefir produced with cow milk. Buffalo milk is mainly used in cream production in Turkey, particularly in Afyonkarahisar region. The residual of this milk, which is used in cream production, is called as the "underside of cream". Producing kefir from "underside of cream" may both increase the economic value of buffalo milk and contribute to the variety of products made from buffalo milk. Although starter culture is used instead of grain to provide standardization in the industrial kefir production, the use of kefir grain could be more appropriate in terms of technological and sensory characteristics of kefir.

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