ePublishing

ΕΘΝΙΚΟ ΚΕΝΤΡΟ ΤΕΚΜΗΡΙΩΣΗΣ
EKT NATIONAL DOCUMENTATION CENTRE

Journal of the Hellenic Veterinary Medical Society

Vol 77, No 2 (2026)

Microbiological changes occurring during storage in kefirs with pomegranate (Punica granatum L.) juicy produced from cow's milk

B Hallaç, H Sancak

doi: 10.12681/jhvms.42107

Copyright © 2026, B Hallaç, H Sancak

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0.

To cite this article:

Hallaç, B., & Sancak, H. (2026). Microbiological changes occurring during storage in kefirs with pomegranate (Punica granatum L.) juicy produced from cow's milk. *Journal of the Hellenic Veterinary Medical Society*, *77*(2), 10513–10522. https://doi.org/10.12681/jhvms.42107

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 24/05/2026 14:49:21

J HELLENIC VET MED SOC 2026, 77 (2): 10513-10522
ΠΕΚΕ 2026, 77 (2): 10513-10522

Research article
Ερευνητικό άρθρο

Microbiological changes occurring during storage in kefirs with pomegranate (*Punica granatum* L.) juicy produced from cow's milk

B. Hallac,[1*] H. Sancak[2]

[1]*Siirt University, Faculty of Engineering, Department of Food Engineering, Siirt, Turkey*

[2]*Bitlis Eren University, Organized Industrial Zone Vocational School, Department of Food Processing, Bitlis, Turkey*

ABSTRACT: This research was conducted to determine the microbiological changes that occur during storage (zeroth, 7th, 14th, 21st, 28th days) in kefirs produced by adding different concentrations of pomegranate (*Punica granatum* L.) juice (6.25%, 12.5%, 25%, 50%) to cow's milk. Sterile cow's milk, kefir grains, and Zivzik pomegranate juice were used in production. The highest total aerobic mesophilic microorganism (TAMM) count (9.00±0.35 $\log_{10}$ CFU/mL) was determined in sample at 50% concentration on the 14th day of storage. In the last stages of storage, TAMM counts decreased at all concentrations due to the inhibitory effect of pomegranate juice on microorganisms. The count of coliform group microorganism determined at 3 $\log_{10}$ CFU/mL levels in all concentrations also partially decreased during storage and *Escherichia coli* wasn't encountered in samples. However, the count of *Lactobacillus* spp. increased due to increasing pomegranate juice concentrations and improved fermentation. A statistically significant relationship ($p<0.01$) was observed between *Lactobacillus* spp. and coliforms determined in kefir samples. During storage, fermentation has shown its effect on kefirs with pomegranate juicy and a significant relationship ($p<0.01$) was determined between yeast and mold count and pomegranate juice concentrations. As a result, it was observed that high concentrations of pomegranate juice in kefirs created an inhibitor effect on microorganisms in a generally. The fact that kefirs with pomegranate juicy comply with the criteria indicated in the Turkish Food Codex Fermented Dairy Products Communiqué and Microbiological Criteria Regulation in terms of *E. coli* shows that the consumption of these products during storage won't pose any risk to public health. It is thought that individuals who have digestive system problems due to the count of *Lactobacillus* spp. detected above the level specified in the Turkish Food Codex Fermented Dairy Products Communiqué and increasing during storage can easily use these products containing probiotics. However, in the samples, a prominently mold growth occurred during the final days of storage and coliform group microorganisms thought to have originated from the kefir grains used in production were detected. Therefore, when quality and hygienic materials are used in production, kefirs with pomegranate juicy that comply with standards and have a shelf life of at least 21 days' can be produced. Considering the taste and antioxidant activity of pomegranate juice, it is thought that kefirs with pomegranate juicy produced at hygienic conditions can be effective in healthy nutrition and these innovative products can contribute to economic development.

Keyword: Kefir; Pomegranate (*Punica granatum* L.); Storage time; Microbiological change

Correspondence author:
Bulent Hallac
Siirt University, Faculty of Engineering, Department of Food Engineering, Siirt, Turkey
E-mail address: bulenthallac@siirt.edu.tr

Date of submission: **12-7-2025**
Date of acceptance: **2-1-2026**

INTRODUCTION

Fermented foods, whose nutritional values are high and which have longer shelf life compared to many foods (Walstra et al., 2006; Lv and Wang, 2009), and particularly kefir, which has recently stood out with its functional properties (contains effective probiotics for the digestive and immune systems) (Lv and Wang, 2009; Güzel Seydim and Kök Taş, 2019), continue to attract people's attention. Kefir is a fermented dairy product that is produced using kefir grains or starter cultures containing lactose-fermenting (*Kluyveromyces marxianus*) and non-lactose-fermenting (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, *Saccharomyces exiguus*) yeasts (Anonymous, 2009). Kefir, which started to be produced from sheep, goat, and cow milk in Russia and Southwest Asia countries, is generally produced from cow's milk today in many regions of the world (Walstra et al., 2006; Lv and Wang, 2009; Güzel Seydim and Kök Taş, 2019). Kefir, which resembled ayran with its sourish taste, is also similar to yoghurt because of its property of probiotics holding on to the intestinal system (Güzel Seydim and Kök Taş, 2019).

In traditional kefir production, after the milk is heated at 85-95°C for 5-20 min, it is cooled down to 20-25°C, which is the fermentation temperature, and kefir grains by 2-10% are added. Kefir grains are removed from this mixture by filtering, which is then left to fermentation for 14-24 h at fermentation temperature, and kefir is kept at 4-8°C (Irigoyen et al., 2005; Lv and Wang, 2009; Turantaş, 2015). It has been reported that kefirs, which is produced under hygienic and appropriate fermentation conditions, can be kept in the cooler part of the fridge (+4°C) for 30 days (Güzel Seydim and Kök Taş, 2019).

The most important phenolic substance in pomegranate (*Punica granatum* L.), which has an antioxidative property, is punicalagin (Seeram et al., 2005). Pomegranate juice has effects such as blocking damage to the veins, preventing prostate cancer and calcinosis, stop diarrhea, protecting cells against autoxidation harms and delaying osteoarthrosis (Malik et al., 2005; Seeram et al., 2005; Lv and Wang, 2009; Fatahi et al., 2021).

Plain and fruit-flavored kefirs are produced by many companies in line with the consumers' preferences. In the researches conducted on fruit-flavored kefirs (Kök Taş et al., 2014; Harmankaya et al., 2019; Alagöz Kabakcı et al., 2020; Hallac and Sancak, 2021; Ozcelik et al., 2021; Paredes et al., 2022) that produced through innovative works according to the palate of consumers and plain kefirs (Irigoyen et al., 2005; Kavas, 2015) many properties of these products have been demonstrated. There are also studies examining the microbiological properties of kefir, which is one of the important beverages and whose consumption continues to increase (Irigoyen et al., 2005; Kavas, 2015; Wulandari et al., 2017; Kim et al., 2018; Say et al., 2019).

Kök Taş et al. (2014) stated that on the 1st, 7th and 14th days of storage, the count of total aerobic mesophilic microorganism (TAMM) was determined in kefirs with plum (10%) as 8.88, 9.22 and 8.94 $\log_{10}$ CFU/mL, and also in kefirs with molasses (7.5%) as 9.19, 9.29 and 9.09 $\log_{10}$ CFU/mL. Say et al. (2019) reported the TAMM count in kefirs with strawberry-flavored (0.15%) containing kefir grains by 2% to be 8.27 $\log_{10}$ CFU/mL on 1st day, 6.78 $\log_{10}$ CFU/mL on 7th day, 6.88 $\log_{10}$ CFU/mL on 14th day, and 6.98 $\log_{10}$ CFU/mL on 21st day, and in kefirs with apricot-flavored (0.15%) to be 8.30, 6.97, 7.83 and 7.07 $\log_{10}$ CFU/mL, respectively.

In some studies on kefirs, coliforms weren't encountered (Sezer, 2003; Kök Taş et al., 2014; Say et al., 2019), and in some studies these microorganisms were detected at different levels (Molska et al., 2003; Dinç, 2008; Karabıyıklı and Daştan, 2016) have been reported. Var et al. (2021) stated that highly concentrated sauces with pomegranate juicy showed more antimicrobial effects on some important pathogenic microorganisms (*Staphylococcus aureus*, *Salmonella* Typhimurium, *Escherichia coli* O157:H7).

Irigoyen et al. (2005) reported that the count of *Lactobacillus* spp., which was determined as 6.40 $\log_{10}$ CFU/mL in kefirs containing 1% kefir grains and 6.20 $\log_{10}$ CFU/mL in kefirs containing 5% kefir grains on the 28th day of storage. Harmankaya et al. (2019) in kefirs, produced as experimentally, reported that *Lactobacillus* spp. (8.544 $\log_{10}$ CFU/mL) and yeast (6.954 $\log_{10}$ CFU/mL) counts were determined the highest in kefir with strawberry on the 2nd day of storage. Alagöz Kabakcı et al. (2020) reported that the count of *Lactobacillus* spp. decreased during storage in anthocyanin-enriched kefirs and the yeast count was determined the least at the 4th week (3.20 $\log_{10}$ CFU/g) in 10% kefir with pomegranate juicy. Kavas (2015) stated that the count of yeast in kefirs produced by adding 2.5% kefir grains to cow's milk, and 10% kefir grains and 1% glucose to camel's milk increased during storage. In another study (Ozcelik et al., 2021), it was noted that the count of yeast

increased on the last day (28th day) of storage in kefirs with cornelian cherry (6 $\log_{10}$ CFU/mL) and hawthorn juicy (6.20 $\log_{10}$ CFU/mL).

This research was carried out to determine the microbiological changes that occur during storage in kefirs with pomegranate juicy different concentrations produced from cow's milk and Zivzik pomegranate, which has antioxidant properties. In addition, this research is important in terms of ensuring that production is carried out in a way that doesn't pose a risk to public health and revealing the shelf life of kefirs with Zivzik pomegranate juicy according to the microbiological changes detected during storage.

MATERIALS AND METHODS

In this research, sterile cow's milk with 1.5% fat content (Icim, Istanbul, Turkey) purchased from the market, kefir grains obtained from the producers in Eskisehir (Turkey), and pomegranate juice obtained from Zivzik pomegranate (*Punica granatum* L.) purchased from producers in Siirt (Turkey) were used. Pomegranate seeds were separated from their peels under aseptic conditions and pressed in a juicer (Kale, 1701, Istanbul, Turkey) to obtain pomegranate juice.

Kefir production and preparation of samples

For traditional kefir production, kefir grains (5%) were added to sterile cow's milk kept at room temperature, and this mixture was fermented at 25°C for 18-24 h in incubator (POL-EKO®, SLN 53, Poland). At the end of this time, kefir grains were filtered using a strainer (Myden, Usak, Turkey), plain kefir as the control group and kefirs with pomegranate juicy obtained in different concentrations (6.25%, 12.5%, 25%, 50%) by adding freshly squeezed Zivzik pomegranate juice under aseptic conditions were produced. All samples were produced twice. Kefirs whose pH values were adjusting at 4.0 with lactic acid (Sigma-Aldrich, 27714, Germany) in sterile glass jars (Pasabahce, Istanbul, Turkey) were kept (+4°C) in cooled incubator (Velp Scientifica, F10300310, Italy), and microbiological analyses were performed on this kefirs during storage (zeroth, 7th, 14th, 21st and 28th days) (Fig 1).

Microbiological analyses

Total aerobic mesophilic microorganism (TAMM) enumeration of kefirs produced at different concentrations were performed on Plate Count Agar (PCA), coliform group microorganism enumeration on Lactose Broth+Eosin Methylen Blue (EMB) Agar, *Escherichia coli* enumeration on EC Broth+EMB Agar,

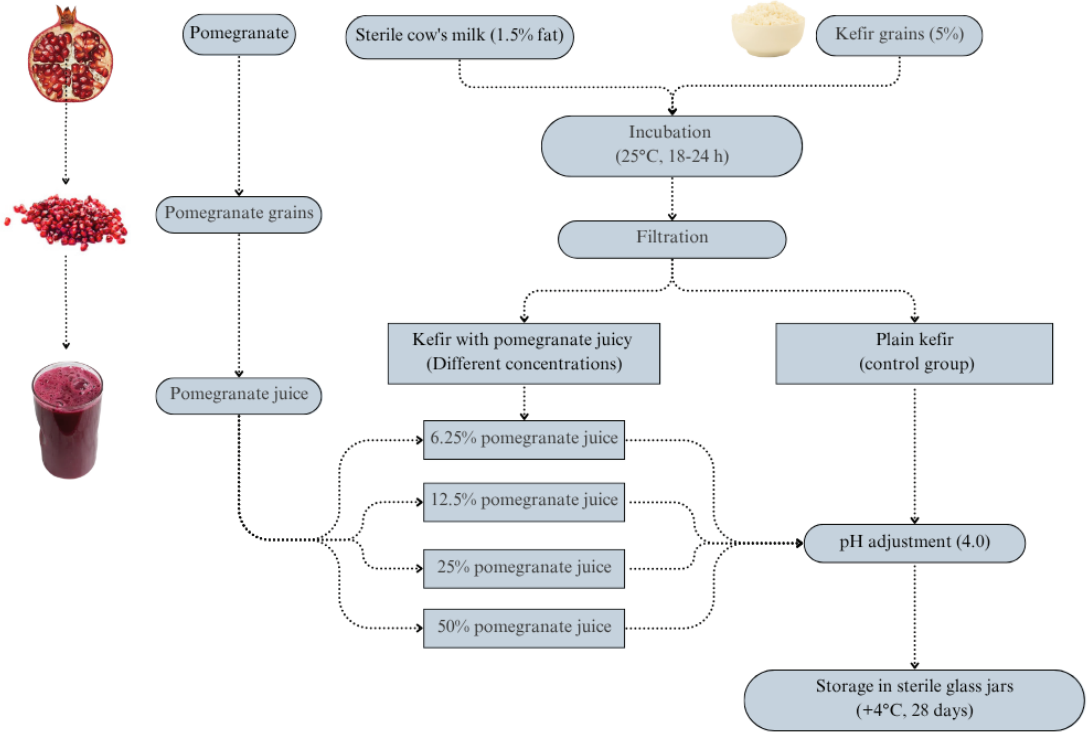

Figure 1. Kefir with pomegranate juicy production process.

Table 1. Media used in microbiological analysis of kefirs, applied cultivation methods and incubation conditions

Microorganism	Medium	Cultivation	Incubation	Reference
TAMM	Plate Count Agar (Oxoid CM0463, UK)	Spreading	30°C 24-48 h (Aerobic)	Anonymous (2013)
Coliforms	Lactose Broth (LAB 126, UK)	Loop	37°C 18-24 h (Aerobic)	Feng et al. (1998)
	Eosine Methylen Blue Agar (Oxoid CM0069, UK)	Spreading	37°C 24 h (Aerobic)	
Escherichia coli	EC Broth (LAB 171, UK)	Loop	44-45.5°C 24-48 h (Aerobic)	Feng et al. (1998)
	Eosine Methylen Blue Agar (Oxoid CM0069, UK)	Spreading	44-45.5°C 24-48 h (Aerobic)	
Lactobacillus spp.	MRS Agar (Merck 110660, Germany)	Spreading	37°C 24 h (Anaerobic)	Harrigan (1998)
Yeast and mold	Potato Dextrose Agar (Oxoid CM0139, UK)	Spreading	25°C 24-48 h (Aerobic)	Harrigan (1998)

TAMM: Total aerobic mesophilic microorganism

Lactobacillus spp. enumeration on MRS Agar and yeast and mold enumeration on Potato Dextrose Agar (PDA). Information on the media used in microbiological analyses, applied cultivation methods, and incubation conditions are presented collectively in Table 1. Analyses were performed in two replicates and three parallels, and the counts of microorganism were evaluated as logarithmic ($\log_{10}$ CFU/mL).

Statistical analyses

The findings were evaluated in the Statistical Package for the Social Sciences (SPSS) 23.0 statistical package software (IBM, USA). The findings, whose mean and standard deviation values (±sd) were determined, were subjected to variance analysis (ANOVA), and Duncan's multiple comparison tests and correlation analyses ($p<0.01$) were applied to determine the differences between the groups (Anonymous, 2015).

RESULTS

TAMM, coliform group microorganism, *Lactobacillus* spp., and yeast and mold counts of the control group and kefirs with pomegranate juicy in different concentrations (6.25%, 12.5%, 25%, 50%) on zeroth, 7th, 14th, 21th and 28th days are presented in Fig 2-5, respectively. Additionally, microbiological analysis findings ($\log_{10}$ CFU/mL) determined in kefirs with

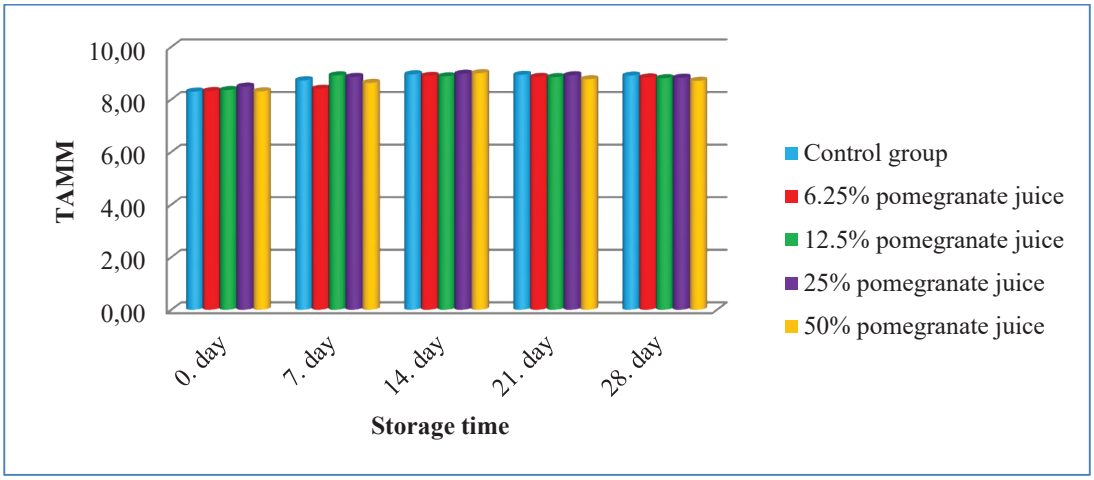

Figure 2. TAMM count depending on concentration and time ($\log_{10}$ CFU/mL).

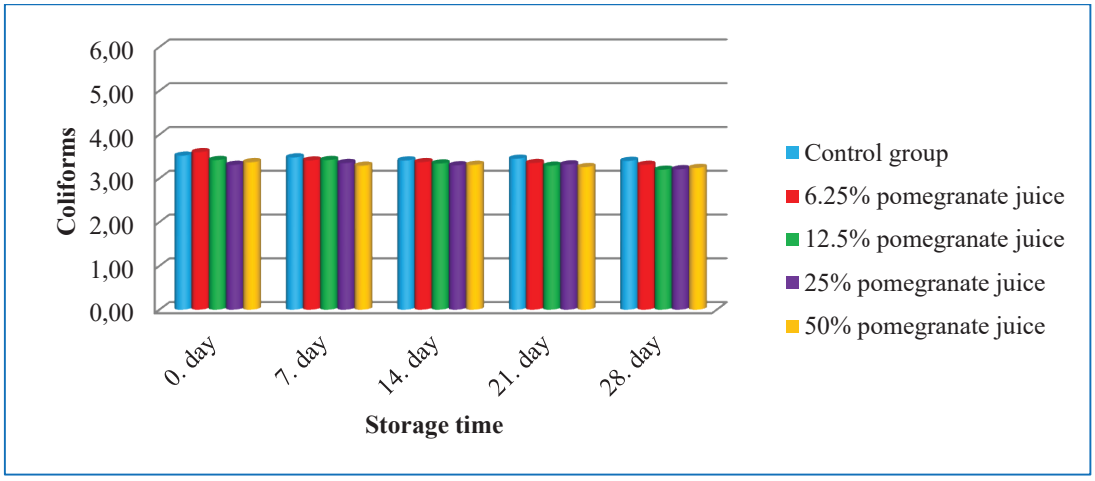

Figure 3. Coliform group microorganism count depending on concentration and time (log_{10} CFU/mL).

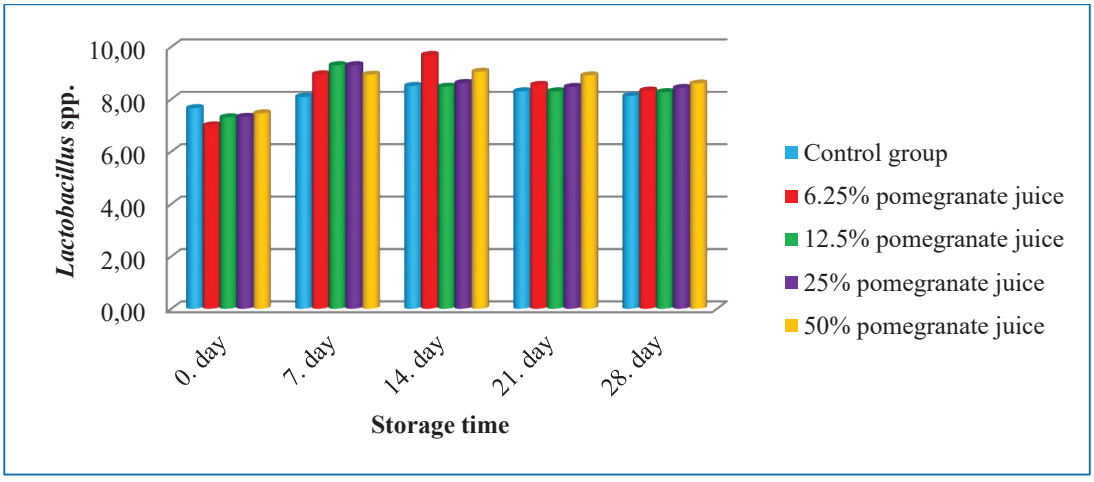

Figure 4. *Lactobacillus* spp. count depending on concentration and time (log_{10} CFU/mL).

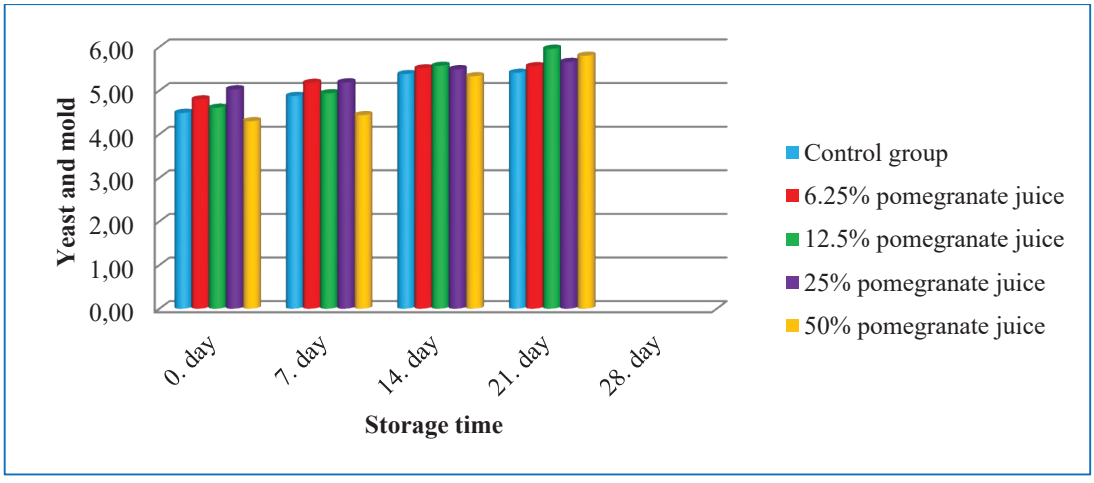

Figure 5. Yeast and mold count depending on concentration and time (log_{10} CFU/mL).

pomegranate juicy and statistical relationships between groups ($p<0.01$) are given in Table 2. *E. coli* wasn't encountered in kefir samples. In this research, examined samples during storage, a statistically significant relationship ($p<0.01$) was found between coliforms and *Lactobacillus* spp. count, and between yeast and mold count and pomegranate juice concentrations.

DISCUSSION

Due to the distinctive taste and flavor of kefir, some consumers are distant from this product. Therefore, with the addition of various fruits to kefir, which has superior functional properties, it becomes easier to drink, their consumption level increases and healthy products that won't deteriorate in a short time can be obtained. In some studies, (Hallac and Sancak, 2021; Ozcelik et al., 2021), it has been reported that kefirs with pomegranate juicy have attracted attention from consumers. It has been stated that the average amounts of caffeic acid, quercetin, catechin and gallic acid, which are antioxidant phenolic compounds in Zivzik pomegranate, were determined as 53 mg/L, 48.5 mg/L, 8.8 mg/L and 3.4 mg/L, respectively (Vardin et al., 2012) and that this pomegranate can be preserved for up to 4-6 months when stored under appropriate conditions (Al-Jabbarı et al., 2019; Dündar and Çağlın, 2023). In this research, the microbiological changes that occur during storage in kefirs produced using Zivzik pomegranate, which

Table 2. Microbiological analysis findings (log_{10} CFU/mL) in kefirs with pomegranate juicy and statistical relationships ($p<0.01$) between groups

	Storage time (Days)				
Concentration	**0**	**7**	**14**	**21**	**28**
			TAMM		
Control	8.30 ± 0.92^{c}	8.73 ± 0.42^{ABb}	8.96 ± 0.28^{ABa}	8.94 ± 0.42^{a}	8.91 ± 0.64^{a}
6.25%	8.32 ± 0.42^{b}	8.41 ± 0.49^{Cb}	8.90 ± 0.35^{BCa}	8.86 ± 0.28^{a}	8.84 ± 1.27^{a}
12.50%	8.36 ± 0.49^{b}	8.92 ± 1.20^{Aa}	8.88 ± 0.35^{Ca}	8.85 ± 1.20^{a}	8.81 ± 0.49^{a}
25%	8.49 ± 0.49^{b}	8.86 ± 1.20^{ABa}	8.98 ± 0.21^{ABa}	8.92 ± 1.48^{a}	8.83 ± 0.78^{a}
50%	8.31 ± 1.34^{c}	8.63 ± 0.78^{Cb}	9.00 ± 0.35^{Aa}	8.77 ± 0.92^{b}	8.71 ± 0.21^{b}
			Coliforms		
Control	3.52 ± 0.64^{AB}	3.48 ± 0.64^{A}	3.41 ± 0.35	3.45 ± 0.92	3.40 ± 1.20
6.25%	3.60 ± 0.78^{Aa}	3.41 ± 0.35^{ABb}	3.37 ± 0.49^{b}	3.35 ± 0.42^{b}	3.31 ± 0.49^{b}
12.50%	3.42 ± 0.21^{AB}	3.42 ± 0.92^{AB}	3.34 ± 0.49	3.29 ± 1.21	3.20 ± 1.20
25%	3.31 ± 1.63^{B}	3.35 ± 0.35^{AB}	3.30 ± 0.21	3.32 ± 0.21	3.21 ± 0.92
50%	3.37 ± 0.21^{AB}	3.29 ± 0.64^{B}	3.31 ± 0.78	3.26 ± 0.28	3.24 ± 0.35
			***Lactobacillus* spp.**		
Control	7.66 ± 0.42^{Ad}	8.09 ± 0.64^{Cc}	8.51 ± 0.92^{Ca}	8.30 ± 1.20^{Cb}	8.12 ± 0.35^{Dbc}
6.25%	7.00 ± 0.49^{Ce}	8.95 ± 0.49^{Bb}	9.69 ± 0.78^{Aa}	8.55 ± 0.49^{Bc}	8.33 ± 0.35^{BCd}
12.50%	7.31 ± 0.92^{Bc}	9.30 ± 1.20^{Aa}	8.48 ± 0.64^{Cb}	8.30 ± 0.92^{Cb}	8.27 ± 0.35^{Cb}
25%	7.33 ± 0.64^{Bd}	9.30 ± 0.49^{Aa}	8.62 ± 0.64^{Cb}	8.47 ± 0.35^{BCc}	8.43 ± 0.35^{Bc}
50%	7.46 ± 0.64^{Bc}	8.94 ± 0.64^{Ba}	9.05 ± 0.49^{Ba}	8.91 ± 0.64^{Aa}	8.60 ± 1.06^{Ab}
			Yeast and mold		
Control	4.49 ± 1.20^{DCd}	4.88 ± 0.78^{Ac}	5.38 ± 0.92^{ab}	5.41 ± 1.20^{Ca}	Intense mold
6.25%	4.80 ± 1.34^{ABc}	5.18 ± 0.92^{Ab}	5.51 ± 1.20^{a}	5.56 ± 0.49^{BCa}	Intense mold
12.50%	4.61 ± 0.49^{BCd}	4.94 ± 2.05^{Ac}	5.57 ± 1.06^{b}	5.96 ± 0.49^{Aa}	Intense mold
25%	5.03 ± 0.78^{Ac}	5.19 ± 0.78^{Ac}	5.49 ± 1.20^{ab}	5.66 ± 1.91^{ABCa}	Intense mold
50%	4.30 ± 0.28^{Dc}	4.44 ± 1.77^{Bc}	5.33 ± 0.92^{b}	5.80 ± 1.20^{ABa}	Intense mold

TAMM: Total aerobic mesophilic microorganism; **^{abcde}:** Indicates the difference between the changes that occur during storage ($p<0.01$); **^{ABC}:** Indicates the difference between the changes that occur depending on the concentrations ($p<0.01$)

has a juicy and pleasant flavor compared to other pomegranates and is generally grown commercially in the Sirvan district of Siirt (Taş, 2015; Al-Jabbarı et al., 2019), were investigated.

While there was a small increase in the TAMM count of kefirs with pomegranate juicy at different concentrations in the early stages of storage, there was a drop in the following days (Fig 2). As a matter of fact, it is thought that this decrease may be due to the antimicrobial effect of pomegranate juice (Var et al., 2021) together with the bioactive substances (Lv and Wang, 2009) formed as a result of fermentation. Kök Taş at al. (2014) reported that the count of TAMM in kefirs with plum and molasses increased until the 7th day of storage and then decreased, while Say et al. (2019) reported that this count started to decrease in the first days of storage in kefirs with strawberry-flavored and apricot-flavored. The report by Kök Taş et al. (2014) indicated that TAMM count examined in kefirs increased until the 7th day of fermentation and decreased in the ensuing days, which is similar to the findings of this research. The high amount of carbohydrates in fruit kefirs causes an increase also in the count of TAMM in the first days of storage due to the rapid growth of *Lactobacillus* spp. and *Bifidobacteria* (Güzel Seydim and Kök Taş, 2019). Say et al. (2019) report that the count of TAMM decreased from the beginning of storage is different from the findings of this research. In studies examining fruit kefirs in Ankara (Turkey), it was reported that the TAMM count was determined at approximately 6-7 $\log_{10}$ CFU/mL (Uslu, 2010) and 7-8 $\log_{10}$ CFU/mL (Dinç, 2008) levels. The maximum TAMM count determined in pomegranate juicy kefir with 50% concentration on the 14th day of storage (9.00±0.35 $\log_{10}$ CFU/mL) was found to be higher than the values reported by Dinç (2008) and Uslu (2010). The differences observed in the TAMM counts of the kefirs examined in the studies during storage may be due to the use of various fruits and flavors in production, the microbiological quality of the kefir grains and their addition ratios to the kefir, as well as the storage times and temperatures of the kefirs.

The coliform group microorganism count determined to be at 3 $\log_{10}$ CFU/mL level in samples examined in this research generally decreased during storage (Fig. 3 Ascorbic acid, flavonoid substances, and water-soluble polyphenols in pomegranate juice (Güzel Seydim and Kök Taş, 2019), and antimicrobial compounds that form during the fermentation of kefir (Lv and Wang, 2009) slows down the development of particularly pathogenic microorganisms. Coliforms were encountered during storage in this research suggests that however pomegranate juice and kefir have an antimicrobial effect on pathogenic microorganisms, it may be due to the microbial load of kefir grains used in production.

In the Turkish Food Codex Fermented Dairy Products Communiqué (Anonymous, 2009) specified that the maximum coliform group microorganism count allowed in kefirs must be at most 95 MPN/mL in two of five samples. In this research, coliforms were detected above the limit specified in the communiqué in all concentrations of kefirs with pomegranate juicy during storage. It is seen that the examined samples aren't suitable in terms of these microorganisms according to the relevant communiqué. It is important to use kefir grains and fruit juices of appropriate hygienic quality, in the production of kefirs which will be sold on the market in order to obtain products that comply with the standards and don't pose potential risks to public health.

Dinç (2008) stated that encountered *E. coli* in the plain, diet and fruit-added kefirs available in the market, while Karabıyıklı and Daştan (2016) encountered this microorganism in industrial kefirs purchased from the market and kefir grains used in production. In this research, while coliforms were determined in samples examined during storage (Table 2), *E. coli* wasn't encountered. In the Turkish Food Codex Fermented Dairy Products Communiqué (Anonymous, 2009) and the Microbiological Criteria Regulation (Anonymous, 2011) specified that the count of *E. coli* that can be found in kefir should be <3 MPN/mL. All samples examined in this research were found to comply with the criteria specified in these legislations in terms of *E. coli*.

In fermented products such as kefir, the amount of lactose decreases while the amount of lactic acid and ethyl alcohol increases depending on the storage time and the activities of microorganisms (Lv and Wang, 2009; Kılıç, 2014; Güzel Seydim and Kök Taş, 2019). Also in this research, the count of *Lactobacillus* spp. in the control group kefirs increased by 0.46 $\log_{10}$ CFU/mL during storage and was determined as 8.12±0.35 $\log_{10}$ CFU/mL on the 28th day (Table 2). In kefirs with pomegranate juicy produced at different concentrations, there was an increase of approximately 1 $\log_{10}$ CFU/mL during storage (Table 2). While the most increase was observed in the 6.25% concentration sample (7.00-8.33 $\log_{10}$

CFU/mL), the highest count of *Lactobacillus* spp. was determined in the 50% concentration sample (7.46-8.60 $\log_{10}$ CFU/mL) (Fig. 4). Witthuhn et al. (2005) reported that in kefirs produced traditionally in South Africa, *Lactobacillus* spp. count was determined 1.6×10^7 CFU/mL end of storage time (30^{th} day). Dinç (2008) reported that *Lactobacillus* spp. count in the fruit kefirs changed was between 7.20-9.14 $\log_{10}$ CFU/mL. The *Lactobacillus* spp. count determined in all concentrations during storage in this research is similar to the findings obtained by Dinç (2008). However, the values determined on the 28^{th} day of storage in all concentrations are higher than the value reported by Witthuhn et al. (2005) on the last day of storage. These differences between studies may be due to the kefir grains used in production and the storage conditions of the kefirs.

Natural antibiotics such as bacteriocins (bifidin, bifidocin) found in the gastro-intestinal system of humans and animals exhibit antimicrobial effect against pathogenic microorganisms and prevent their development (Lv and Wang, 2009; Ünlütürk et al., 2015). However, in this research, a significant relationship ($p<0.01$) has been seen between *Lactobacillus* spp. count and coliforms determined in kefirs. It is thought that the possible microbial load of kefir grains used in production causes the determined of coliforms.

Probiotic foods, which have antimicrobial properties, play an important role in healthy nutrition and their regular consumption is recommended (Lv and Wang, 2009; Turantaş, 2015; Guzel-Seydim et al., 2021). In the Turkish Food Codex Fermented Dairy Products Communiqué (Anonymous, 2009) stated that count of the *Lactobacillus* spp. can be included in kefirs must be at least 10^7 CFU/mL. It is seen that kefirs examined during storage in all concentrations in this research conformed to the relevant communiqué in terms of *Lactobacillus* spp. count. Kefirs with pomegranate juicy, which also have probiotic properties and a taste that can attract the attention of consumers, can easily used by individuals who have digestive system problems.

The yeast and mold count in kefirs with Zivzik pomegranate juicy generally increased as the storage time was prolonged (Fig. 5). Yeasts play an important role in creating the unique aroma of kefir by creating organic compounds such as lactic acid, carbon dioxide and ethyl alcohol (Lv and Wang, 2009; Güzel Seydim and Kök Taş, 2019). A statistically significant relationship ($p<0.01$) was determined between the yeast and mold count determined in kefirs examined in this research and the pomegranate juice concentrations used in production. This relationship can be explained by the increase in yeast and mold counts due to the increase in pomegranate juice concentrations.

Karabıyıklı and Daştan (2016) reported that yeast count in the industrial kefirs they examined was between <1.00-5.68 $\log_{10}$ CFU/mL and in traditional kefirs between 1.56-5.82 $\log_{10}$ CFU/mL, and they stated that no mold was encountered in any samples. Mantzourani et al. (2019) reported that they were encountered yeast and mold during the 28-day storage in pomegranate juice utilizing *Lactobacillus plantarum* ATCC 14917 strain. In this research, the yeast and mold count determined between 4-5 $\log_{10}$ CFU/mL at all concentrations during storage is lower than the values (5-9 $\log_{10}$ CFU/mL) reported in experimentally produced kefirs (Sezer, 2003). The difference determined in the studies conducted in terms of yeast and mold count may have originated from production hygiene, kefir grains used in the production, fruit ratios with microbiological qualities of these, the storage temperature and time of kefirs, and media used in the analyses and their pH values. In addition, it is thought that adjusting the initial pH levels of the kefirs produced in this research to 4.0 is effective in determining this count at different levels.

It has been stipulated in the Turkish Food Codex Fermented Dairy Products Communiqué (Anonymous, 2009) that the minimum yeast count in kefir must be 10^4 CFU/mL, and the maximum mold count must be 10^3 CFU/mL in two of five samples. In the samples examined in this research, visible mold growth occurred on the 27^{th} and 28^{th} days of storage (Table 2). When yeast and mold count determined in kefirs at all concentrations during storage was evaluated together, it is seen that only samples examined in terms of yeast count are in accordance with the relevant communiqué (Anonymous, 2009).

CONCLUSIONS

As a result, it was observed that 25% and 50% kefirs with pomegranate juicy showed more antimicrobial effects towards the last stages of storage. The absence of *E. coli* in kefirs with pomegranate juicy at different concentrations and the high levels of *Lactobacillus* spp. count showed that these kefirs comply with the legislations. Individuals who experience digestive system problems can comfortably

use these products, which have high *Lactobacillus* spp. counts due to increased pomegranate juice concentrations and therefore contain probiotics. However, in this research coliform group microorganisms thought to originate from kefir grains used in production were detected in samples examined and visible mold growth was also observed in the last stages of storage. Therefore, when production is carried out using hygienic materials and preventing all contamination, kefirs with pomegranate juicy can be produced that don't pose a health risk and have a shelf life of at least 21 days'. Due to the taste of pomegranate juice that is appreciated by consumers and its antimicrobial effect on microorganisms, kefirs with pomegranate juicy produced by taking hygienic measures can be used in healthy nutrition. In addition, these innovative products can contribute to economic development by creating new employment sources.

ACKNOWLEDGMENTS

We would like to thank Siirt University Scientific Research Projects Coordinatorship (2018-SİÜ-MÜH-028), who provided financial support for the Project.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

REFERENCES

Alagöz Kabakcı S, Türkyılmaz M, Özkan M (2020) Changes in the quality of kefir fortified with anthocyanin-rich juices during storage. Food Chemistry 326 (126977):1-11. https://doi.org/10.1016/j.foodchem.2020.126977

Al-Jabbarı KH, Pakyürek M, Yaviç A (2019) Identification of morphological and pomological characteristics of Iraq pomegranate (*Punica granatum* L.) variety Salakhani and comparing with variety Zivzik. International Journal of Secondary Metabolite 6 (3):270-282. https://doi.org/10.21448/ijsm.598508

Anonymous (2009) Turkish Food Codex Communique on Fermented Milk Products. Official newspaper: February 16, 2009, 27143, Ankara, Turkey.

Anonymous (2011) Turkish Food Codex Regulation on Microbiological Criteria. Official newspaper: December 29, 2011, 28157 (3rd Repetitive), Ankara, Turkey.

Anonymous (2013) Microbiology of the Food Chain-Horizontal Method for the Enumeration of Microorganisms. Part 2: Colony Count at 30°C by the Surface Plating Technique. International Organization for Standardization (ISO 4833-2: 2013).

Anonymous (2015) IBM SPSS Statistics for Windows, Version 23.0. IBM Corp, Armonk, New York, USA.

Dinç A (2008) Determination of Some Microbiological and Chemical Properties of Kefir. MsC Thesis, University of Ankara, Institute of Health Sciences, Department of Food Hygiene and Technology, Ankara, Turkey.

Dündar Ö, Çağlın E (2023) The comparison of postharvest storage of Hicaznar and Zivzik pomegranate in the Southeastern Anatolia Region. Recep Tayyip Erdogan University Journal of Science and Engineering 4 (1):1-8. https://doi.org/10.53501/rteufemud.1177613

Fatahi A, Soleimani N, Afrough P (2021) Anticancer activity of kefir on glioblastoma cancer cell as a new treatment. International Journal of Food Science 2021 (8180742):1-5. https://doi.org/10.1155/2021/8180742

Feng P, Weagant SD, Grant MA, Burkhardt W (1998) Enumeration of *Escherichia coli* and the coliform bacteria. In: Food and Drug Administration (FDA), Bacteriological Analytical Manual (BAM) Online, 8th ed, Chapter 4, Silver Spring, Berlin, Germany.

Some properties of kefir enriched with apple and lemon fiber

Guzel-Seydim ZB, Gökırmaklı Ç, Greene AK (2021) A comparison of milk kefir and water kefir: Physical, chemical, microbiological and functional properties. Trends in Food Science &Technology 113 (2021):42-53. https://doi.org/10.1016/j.tifs.2021.04.041

Güzel Seydim ZB, Kök Taş T (2019) Kefir. In: Fermente Gıdalar: Mikrobiyoloji, Teknoloji ve Sağlık, Ed, Anlı E, Şanlıbaba P, 1st ed, Chapter 8, Nobel Academic Publishing Education Consulting Trade Co Ltd, Ankara, Turkey: pp 233-255.

Hallac B, Sancak H (2021) The effect of Zivzik pomegranate juice on the physico-chemical and sensorial properties of kefir produced by conventional method. BEU Journal of Science 10 (1):16-23. https://doi.org/10.17798/bitlisfen.79781

Harmankaya S, Gülbaz G, Kamber U (2019) Microbiological, chemical and sensory characteristics of kefir prepared with various fruit additives. Van Veterinary Journal 30 (1):13-18.

Harrigan WF (1998) Laboratory Methods in Food Microbiology. 3rd ed, Academic Press Limited, London, UK.

Irigoyen A, Arana I, Castiella M, Torre P, Ibáñez FC (2005) Microbiological, physicochemical, and sensory characteristics of kefir during storage. Food Chemistry 90 (4):613-620. https://doi.org/10.1016/j.foodchem.2004.04.021

Karabıyıklı Ş, Daştan S (2016) Microbiologic profile of kefir which is a traditional and functional food. Journal of Agricultural Faculty of Gaziosmanpasa University 33 (1):75-83. https://doi.org/10.13002/jafag919

Kavas G (2015) Kefirs manufactured from camel (*Camelus dramedarius*) milk and cow milk: Comparison of some chemical and microbial properties. Italian Journal of Food Science 27 (3):357-365. https://doi.org/10.14674/1120-1770/ijfs.v279

Kılıç S (2014) Süt Endüstrisinde Laktik Asit Bakterileri. Ege University Faculty of Agriculture Publications, No 42, 3th ed, Ege University Press, Izmir, Turkey.

Kim DH, Jeong D, Song KY, Seo KH (2018) Comparison of traditional and backslopping methods for kefir fermentation based on physicochemical and microbiological characteristics. LWT-Food Science and Technology 97 (11):503-507. https://doi.org/10.1016/j.lwt.2018.07.023

Kök Taş T, İlay E, Öker A (2014) Determination of some quality criteria of the kefir produced with molasses and plum. Turkish Journal of Agriculture-Food Science and Technology 2 (2):86-91. https://doi.org/10.24925/turjaf.v2i2.86-91.82

Lv J, Wang LM (2009) Bioactive components in kefir and koumiss. In: Bioactive Components in Milk and Dairy Products, Ed, Park YW, Chapter 10, Wiley-Blackwell, Singapore: pp 251-262.

Malik A, Afaq F, Sarfaraz S, Adhami VM, Syed DN, Mukhtar H (2005) Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. Proceedings of the National Academy of Sciences of the USA 102 (41):14813-14818. https://doi.org/10.1073/pnas.0505870102

Mantzourani I, Kazakos S, Terpou A, Alexopoulos A, Bezirtzoglou E, Bekatorou A, Plessas S (2019) Potential of the probiotic *Lactobacillus plantarum* ATCC 14917 strain to produce functional fermented pomegranate juice. Foods 8 (1):4. https://doi.org/10.3390/foods8010004

Molska I, Nowosielska R, Frelik I (2003) The changes in microbiological quality of kefir and yoghurt on the Warsaw market in the years 1995-2001. Roczniki Państwowego Zakladu Higieny 54 (2):145-152.

Ozcelik F, Akan E, Kinik O (2021) Use of cornelian cherry, hawthorn, red plum, roseship and pomegranate juices in the production of water kefir beverages. Food Bioscience 42 (101219):1-9. https://doi.org/10.1016/j.fbio.2021.101219

Paredes JL, Escudero-Gilete ML, Vicario IM (2022) A new functional kefir fermented beverage obtained from fruit and vegetable juice: Development and characterization. LWT 154 (112728):1-10. https://doi.org/10.1016/j.lwt.2021.112728

Say D, Tangüler H, Güzeler N (2019) Change of microbiological properties of strawberry and apricot flavored kefir during storage. Academic Platform Journal of Engineering and Science, 7 (2):306-311. https://doi.org/10.21541/apjes.474916

Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D (2005) In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. The Journal of Nutritional Biochemistry 16 (6):360-367. https://doi.org/10.1016/j.jnutbio.2005.01.006

Sezer Ç (2003) Kefirde Laktik Asit Bakterilerinin Tür Düzeyinde Araştırılması. MsC Thesis, University of Kafkas, Institute of Health Sciences, Department of Food Hygiene and Technology, Kars, Turkey.

Taş L (2015) Adoption and Diffusion of Organic Zivzig Pomegranate in Şirvan District of Siirt Province. MsC Thesis, University of Kahramanmaras Sutcu Imam, Institue of Sciences, Department of Agricultural Economics, Kahramanmaras, Turkey.

Turantaş F (2015) Fermente gıdalar. In: Gıda Mikrobiyolojisi, Ed, Ünlütürk A, Turantaş F, 4th ed, Chapter 5.19, Meta Printing Services, Bornova, Izmir, Turkey: pp 447-473.

Uslu G (2010) A Study on Microbiological, Physical, Chemical and Organoleptic Properties of Kefirs, Sold in Ankara Market. MsC Thesis, University of Ankara, Institute of Science, Department of Food Engineering, Ankara, Turkey.

Ünlütürk A, Karapınar M, Turantaş F (2015) Gıdalarda önemli mikroorganizmalar. In: Gıda Mikrobiyolojisi, Ed, Ünlütürk A, Turantaş F, 4th ed, Chapter 1.2, Meta Printing Services, Bornova, Izmir, Turkey: pp 11-43.

Var I, Uzunlu S, Değirmenci I (2021) Antimicrobial effects of fruit sauces on some pathogenic bacteria in vitro and on chicken breast meat. Journal of the Hellenic Veterinary Medical Society 72 (1):2703-2712. https://doi.org/10.12681/jhvms.26755

Vardin H, Karaaslan M, Yılmaz FM, İzol G, Cesur Ö, Yüksekkaya S, Çevik GB (2012) Zivzik ve Görümlü Narlarının Özelliklerinin ve Katma Değerli Ürünlere İşlenebilirliğinin Belirlenmesi. Dicle Development Agency (DIKA), Grafinet Media, Sanlıurfa, Turkey.

Walstra P, Wouters JTM, Geurts TJ (2006) Fermented milks. In: Dairy Science and Technology, Ed, Walstra P, Wouters JTM, Geurts TJ, 2nd ed, CRC Press, Boca Raton, USA: pp 551-573.

Witthuhn RC, Schoeman T, Britz TJ (2005) Characterisation of the microbial population at different stages of kefir production and kefir grain masscultivation. International Dairy Journal 15 (4):383-389. https://doi.org/10.1016/j.idairyj.2004.07.016

Wulandari AO, Purwadi P, Jaya F (2017) Coffee flower honey (*Coffea* sp.) addition to kefir quality based on microbiology characteristic. Jurnal Ilmu dan Teknologi Hasil Ternak 12 (2):83-88. https://doi.org/10.21776/ub.jitek.2017.012.02.4