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Improving Functional Properties of Kefir Produced with Cow and Goat Milk

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ABSTRACT

The purpose of this study was to research some properties of kefir that was obtained from the 1% (w/v) and 2% (w/v) inulin addition to cow-goat milk mixture. In this present study, changes of titrable acidity, pH value, total mesophilic aerobic bacteria, *Lactobacillus* spp., *Lactococcus* spp. and yeast counts of samples in storage were determined. Additionally, samples' total fat content, total solid and viscosity values were reported and taste, consistency, and total acceptance of samples were evaluated. Control group, 1% (w/v) and 2% (w/v) inulin added samples' total solid and fat content, viscosity, pH and titrable acidity (equivalent to lactic acid %) values were investigated and found at the range of; 11.84 – 13.53, 4.4 – 4.8, 365.8 – 488.7, 4.45 – 4.53, 0.80 – 0.84, respectively. On the 40th day of the storage total mesophilic aerobic bacteria, *Lactobacillus* spp *Lactococcus* spp. and yeasts were determined as 10.50-10.55, 10.24-10.58, 10.25-10.58 and 7.60-7.93 log cfu ml^{-1}, respectively.

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İnek ve Keçi Sütleriyle Üretilen Kefirin Fonksiyonel Özelliklerinin Geliştirilmesi

ÖZET

Bu araştırmada % 1 (w/v) ve % 2 (w/v) inülin ilavesiyle üretilmiş inek-keçi sütü kefirlerindeki fiziksel, kimyasal ve mikrobiyal özellikleri araştırılmıştır. Bu çalışmada tüm örneklerde titrasyon asitliği, pH değeri, toplam mezofilik aerobik bakteri sayısı, *Lactobacillus* spp., *Lactococcus* spp. ve maya sayıları depolama süresi boyunca tespit edilmiştir. Aynı zamanda örneklerin toplam yağ, kurumadde ve vizkozite değerleri raporlanmıştır. Tat, yoğunluk ve toplam kabul edilebilirlikdeğerlendirilmiştir. Kontrol grup, %1 (w/v) ve % 2 (w/v) inülin ilaveli gruplar için toplam kurumadde ve yağ miktarı, vizkozite, pH ve titrasyon asitliği (%1 laktik asit eşdeğeri) miktarları sırasıyla 11.84 – 13.53, 4.4 – 4.8, 365.8 – 488.7, 4.45 – 4.53, 0.80 – 0.84 olarak belirlenmiştir. Depolamanın 40. gününde, toplam mezofilik aerobik bakteri sayısı 10.50-10.55, *Lactobacillus* spp. 10.24-10.58, *Lactococcus* spp. 10.25-10.58 ve mayalar 7.60-7.93 log kob ml^{-1} olarak tespit edilmiştir.

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INTRODUCTION

Fermented dairy products are highly consumed all around the world (Gaware et al., 2011; Rotar et al., 2015). The dairy industry is globally expanding, and some functional milk products are particularly preferred by consumers for their positive health effects. Among these dairy products, kefir is known to be an acidic fermented milk product, originated in the Caucasus area and mostly popular in Russia, North-Eastern Europe and Southwest Asia locations (Leite et al., 2013; Oliveira et al., 2017; Lima et al., 2018). Kefir grains are composed of kefiran which is a kind of polysaccharide containing D-glycose and D-galactose (Guzel-Seydim et al., 2005; Turan and Ilter, 2007). Kefir's chemical composition depends not only on the starter-kefir grains but also on its geographical origin, the temperature, and time-related conditions of fermentation, and especially on the type and volume of the milk used. Traditional Kefir is obtained from starter culture called 'kefir grains' which is a semi-hard granule that consists of several lactic acid bacteria and probiotics (Wang et al., 2017). Kefir grain microflora comprises *Lactobacillus* spp. (dominantly; *Lactobacillus acidophilus*, *Lb. lactis, Lb. casei*, *Lb. kefir* and *Lb. delbrueckii* subsp. *bulgaricus*) *Streptococcus lactis*, *S. cremoris*, *Leuconostoc* spp. acetic acid microorganisms (*Acetobacter aceti*, *A. rasens*) and mainly some yeasts (*Candida kefir*, *Saccharomyces cerevisiae*, *Kluyveromyces fragilis*. Thus, kefir is known to be a good source of probiotic microorganism with potential health benefits (Santos et al., 2003; Kok-Tas et al., 2010).

Kefir is made from various types of milk (cow, goat, camel, buffalo, or mare), and is usually produced by mixing two types of milk to enhance its benefits, flavour, and texture, and subjected to secondary fermentation or the addition of additives such as inulin to improve the final product properties (Farag et al., 2020). Goat milk has higher nutrient contents than that of cow's milk (Vitamin A, Vitamin B1 and B2) and it can be digested more easily with the 3.49 µm size fat globules and higher amounts fatty acids (short chain). In addition to that, goat's milk contains caproic, caprilic and capric fatty acids that reduce serum cholesterol content in metabolism. Goat milk has less allergenic properties than cow's milk and its proteins are more easily degraded and absorbed in gastrointestinal system (Ahmed et al., 2015). It is notable that goat's milk is widely consumed for health purposes such as its antiallergenic effect (Haenlein, 2004). Technologically, goat's milk has also some good properties as compared with cow's milk; such as small size fat particles which provide a smoother texture in products, containing low quantity of αs1-casein results soft gel products, as well as higher water binding potential (Gomes et al., 2013).

Probiotic microorganisms and lactic acid bacteria in fermented products show beneficial effects on health if they are consumed adequately. Basically, prebiotics are food ingredients that increase the viability of useful microorganisms in host's metabolism. Inulin, commercially produced from chicory's roots in Belgium and Netherland in the early 1990's, is one of the prebiotics that can be used for this purpose (Yabancı, 2010). Inulin is a non-digestible oligosaccharide with prebiotic property, and it has been successfully applied to well-known dairy products. It is a storage material present in many plants such as wheat, onion and bananas; however, chicory is one of the main raw materials used for industrial production. One of the most important advantages of inulin and certain non-digestible oligosaccharides is their ability for selective stimulation of the bifidobacteria growth in the colon (Glibowski and Zielinska, 2015). Consumers are demanding for foods with increasingly properties, such as pleasant flavor, low calorie value or low-fat content and beneficial health effects (Goncu et al., 2017). In order to improve nutraceutical benefits of kefir, an appropriate approach could involve the enrichment with suitable components able to confer to the drink specific and valuable properties (Aiello et al., 2020). However, there has been limited research conducted on the products fermented with goat's milk. Inulin is generally used to modify the texture, viscosity and sensorial properties of dairy products (Tratnik et al., 2006; Moatsou and Park, 2017).

It is remarked that inulin can increase *Lactobacillus* and *Bifidobacterium* spp. in yoghurts (Oliveira et al., 2012). It is proved that inulin supplementation not only has conservation effect on activity and viability of some *Lactobacillus* strains (casei and acidophilus) but also it decreases the generation time of *Streptococcus* and *Lactobacillus*, significantly (Moghadam et al., 2019). As shown in Birkett and Francis' (2010) study, fructo-oligosaccharides (FOS) can support the growth of *Lactobacillus* and *Bifidobacterium* species, but other microorganisms such as *Escherichia coli* and *Clostridium difficile* do not metabolize the FOS. In inulin-added dairy products, there has been an increase in rheological properties especially water binding capacity and dry matter content.

The objectives of this research were to:

1) Produce functional traditional fermented product kefir and determine the effects of addition 1% (w/v) and 2% (w/v) inulin to cow and goat milk mixture on the survival of total mesophilic aerobic bacteria, *Lactobacillus* spp., *Lactococcus* spp. and yeast counts.
2) Examine some quality parameters such as pH, viscosity values and sensory properties of inulin added kefir and control samples over the course of 40 days of cold storage.

MATERIALS and METHOD

Kefir production

Goat's milk has solitary sensorial characteristics as standard and definite 'goaty' aroma. As some buyers do not like the taste of goat's milk, cow and goat milks were mixed (1:1 v/v) in kefir production. Cow and goat raw milks were obtained from a farm and pasteurized at 85 °C for 10 min. Kefir granules were purchased from market and inulin (Orafti, HPX) was provided from company Artisan Food (Istanbul). Kefir production steps can be seen in Figure 1. Trial groups' names are coded as A, B and C for 1% w/v, 2% w/v inulin added groups and control samples, respectively.

Chemical and physical analyses

An acidity indicator pH was determined using a pH meter (Sartorious PT-15). The dry matter, titrable acidity and fat amounts of samples were measured according to A. O. A.C procedures (Anonymous, 2006). Viscosities were tested with Brookfield DV viscosimeter (11, Pro Extra Model).

Cow and Goat milk' s mixture (1:1 v/v)

⬇

Pasteurized at 85 °C for 10 min

⬇

Cooled to 25 °C
Adding kefir granules (2% w/v) +Inulin (Orafti HPX, Artisan, Istanbul) 1% and 2% (w/v)

⬇

Fermentation at 25 °C 18 h (Until pH 4.6) (in closed glass-jars)

⬇

Kefir grains were aseptically separated

⬇

Kefir samples were stored in glass jars at 4 °C for 40 days

⬇

Analysis were carried out on days; 1, 4, 7, 14, 21 and 40.

Figure 1. Production of cow and goat milk kefir with inulin addition
Şekil 1. İnülin ilaveli inek-keçi sütü kefirlerinin üretimi

Microbiological analyses

Ten ml of kefir samples were diluted with 90 ml of 0.1% (w/v, pepton) sterile water and decimal dilutions were prepared in 9 ml of 0.1% (w/v, pepton) sterile water. Lactic acid bacteria numbers were determined by pour plate technique and counted on de Man Rogosa Sharpe agar (MRS Merck 1.10660.0500) under anaerobic conditions at 37°C/72 h. Total mesophilic aerobic microorganisms were detected on plate count medium (PCA, Merck 1.05463.0500) and incubated at 28-30°C/48 h. *Lactococcus* spp. were counted on M17 plates (Merck 1.15108.0500) using pour plate technique after the incubation at 37°C/48 h in anaerobic conditions. Then, yeasts were enumerated on yeast extract glucose chloramphenicol plates (YGC, Merck 1.0375.0500) and plates were incubated at 25°C/5 days (Halkman and Kayhan, 2000).

Sensorial analyses

Sensory evaluation was conducted by using 5 trained panellists (age 18-40) in Balikesir University. The samples were served in 100 ml portions at about 8 °C. The kefir samples were examined and tested by the panellists who were asked to rate the samples sensorially by using marks on a full-score levels in terms of the flavour, odour, colour and texture quality parameters (0-1; it is not consumed as a human food, 2: unpleasant, 3: mildly 4: good, 5: very good).

Statistical Analyses

SPSS 19.0 software for windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) test was performed to determine mean differences between the A, B and C sample groups. The level of significance between the means was obtained by the Tukey HSD and LSD tests.

RESULTS and DISCUSSION

The average percent dry matter % ±S.D. without fat for cow's milk and goat's milk was 7.93 %±0.18, 8.53 ±0.21, and the fat content % ±S.D. was 3.5± % 0.15, 4.5± % 0.10, respectively.

Average % dry matter contents ± S. D. of A, B and C samples were 13.53 ± 0.04, 13.22 ± 0.04, 11.84 ± 0.1; % fat contents ± S. D. of A, B and C samples were 4.6± 0.12, 4.8± 0.18 and 4.4± 0.14, respectively.

Raw cow and goat milks analyses results were compatible with Turkish Food Codex Raw Milk standards. Guneser and Karagul-Yuceer (2010) also determined 3.25% ± 0.05 fat content averagely and between 10.49%± 0.01 - 15.49% ± 0.19 dry matter contents for goat's milk samples collected from Canakkale region. In the study differences between the dry matter contents % and fat contents % of the samples were of importance when compared with the control groups. Dry matter contents% and fat contents of the samples were not changed during the storage.

Viscosity is a parameter that is directly related with the texture of product and a factor for consumer's preference (Gomes et al., 2013). In the research, during the storage viscosity average values were determined as 488.7± 0.50, 365.8±0.43, 380.1±0.50 cP±S.D. for A, B and C samples, respectively. In the present research, it was found that 1% (w/v) inulin added kefir samples have higher viscosity values than the others. The incorporation of inulin caused an increase in the viscosity of the synbiotic yoghurt drink samples in Soh et al. (2021) study. Also, it was stated that inulin has unique ability to form a discrete highly stable particle gels and contribute to the rheological and textural properties of foods. In a similar research, inulin demonstrated the highest rheological and sensory performance as well as the best viability of probiotics in synbiotic fermented milk (Ozturkoglu-Budak et al., 2019). Helal et al. (2018) found yogurt apparent viscosity increased with inulin addition till 2% and was comparable to full-fat yogurt, the addition of inulin has significantly affected the yogurt viscosity resulted in increasing the viscosity value with the inulin addition. Guven (2005) and Tratnik et al. (2006) put forth that inulin addition (2% w/v) in kefir samples have higher viscosities than the control groups. Also, Iriyogen et al. (2005) found 179-501 cP viscosity values for kefir samples in their research. It was stated that increasing the kefir granules ratio in kefir leads to higher viscosity in kefir samples. It can be explained that total dry matter, protein, fat contents (casein and serum protein ratio), heat process, serum protein denaturation, homogenisation, salt stability of milk, starter culture activities, storage temperature may have an impact on the viscosity of the product (Uslu, 2010).

In the study, the titrable acidity values showed an increasing trend. And the pH values of kefir samples were on a decrasing. In the literature there are many research that describe the effect of pH on viability of probiotic viability. Changes in lactic acid values in inulin added samples were found significantly important ($p<0.05$). Nevertheless, differences for control samples were not found significantly important during the storage ($p>0.05$). Guneser and Karagul-Yuceer (2010) found 0.73-0.79 lactic acid contents in kefir samples produced from different ratio of cow and goat milks mixtures.

In the present research, pH values of samples were determined as between 4.45-4.62. Changes in pH values were not found significant ($p>0.05$). It can be seen in Table 1.

Table 1- Lactic acid% ±S.D. and pHvalues of kefir samples during the +4°C storage
Çizelge 1- Kefir örneklerinin +4°C'de depolamada % laktik asit ±S.D. ve pH değerleri

	A		B		C	
Storage days	**L. a.%**	**pH**	**L. a.%**	**pH**	**L. a. %**	**pH**
1.	$0.63 \pm 0.13^{a*}$	4.62 ± 0.23^{a}	0.65 ± 0.14^{a}	4.54 ± 0.13^{a}	0.62 ± 0.09^{a}	4.61 ± 0.28^{a}
4.	0.70 ± 0.14^{a}	4.47 ± 0.29^{a}	0.70 ± 0.26^{a}	4.48 ± 0.13^{a}	0.69 ± 0.23^{a}	4.49 ± 0.34^{a}
7.	0.67 ± 0.14^{ab}	4.59 ± 0.14^{a}	0.70 ± 0.12^{ab}	4.61 ± 0.13^{a}	0.68 ± 0.20^{a}	4.61 ± 0.13^{a}
14.	0.71 ± 0.14^{bc}	4.53 ± 0.10^{a}	0.72 ± 0.10^{b}	4.52 ± 0.13^{a}	0.71 ± 0.09^{a}	4.54 ± 0.13^{a}
21.	0.78 ± 0.20^{c}	4.46 ± 0.01^{a}	0.75 ± 0.06^{bc}	4.48 ± 0.13^{a}	0.75 ± 0.18^{a}	4.51 ± 0.04^{a}
40.	0.84 ± 0.05^{c}	4.45 ± 0.13^{a}	0.80 ± 0.14^{c}	4.48 ± 0.13^{a}	0.80 ± 0.23^{a}	4.53 ± 0.15^{a}

*Means ±SD within each row not sharing the same lowercase letters are statistically different ($p < 0.05$).

In another research, the pH values of inulin and kefir culture added yoghurt samples were determined as between 4.40-4.70 (Okur et al., 2008). Likewise, Glibowski and Kowalska (2012) determined pH values between 4.47-4.53 after the 24 hours' fermentation in inulin added kefir samples. Agata and Jan (2012) produced fermented goat milk beverage with *Lactococcus lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *Saccharomyces fragilis* culture and they observed 4.57-4.63 pH values changes in samples.

Total mesophilic aerobic bacteria count increased during the storage days but in the control samples there was a drop in bacteria numbers on the 14th day. Increase in bacteria numbers was not found significantly important ($p>0.05$). In other words, for all the sample groups, bacteria numbers were found very close to each other on the 40th days of storage (Fig. 2a)

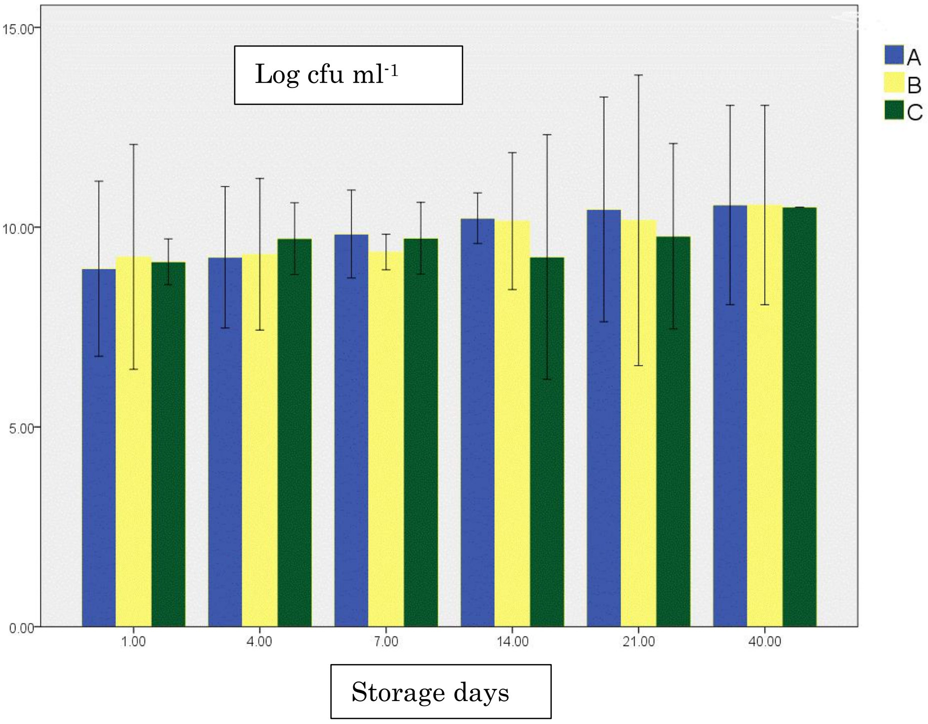
In Uslu (2010) study, mesophilic aerobic bacteria numbers were found 6.41 log cfu/ml in commercially sold kefirs in Ankara markets. Similarly, Karabiyikli and Dastan (2016) determined 7.91-8.50 log cfu/ml and 6.12-7.24 log cfu/ml total mesophilic bacteria in produced kefir samples and commercially sold kefir, respectively.

The highest *Lactobacillus* spp. count were determined in 2% inulin added samples with 11.17 log cfu/ ml on the 21st day. It was observed minimum of 8.55 log cfu/ ml of *Lactobacillus* spp. in control groups on the 1st day of storage. In the study, changes in bacteria counts on the 4 th and 14 th days of storage for 1% inulin added samples, 4 th, 14 th and 40th days of storage for 2% inulin added samples and 4 th, 7 th and 21st days of storage for control samples were found significantly important ($p<0.05$) (Fig. 2b). Similarly in an onoter study, the viability of *L. delbrueckii* ssp. *bulgaricus* was increased by the addition of 1 and 2% of inulin, while the addition of 3% had negative effect. However, no effect was reported in case of *Streptococcus thermophiles* viability in low fat yoghurt samples during the 14 days of storage (Helal et al., 2018). In another study, inulin, added as a prebiotic, increased acidity, as well as enhanced survival of LAB in yogurt-like plant milk fortified with inulin (at 6 °C for 21 days storage) (Łopusiewicz et al., 2020). In a study it was investigated the effects of inulin on some properties of cow milk kefir and goat milk kefir. *Lactobacilli* and *Streptococci* count in goat milk kefir were almost similar to the cow milk kefir.

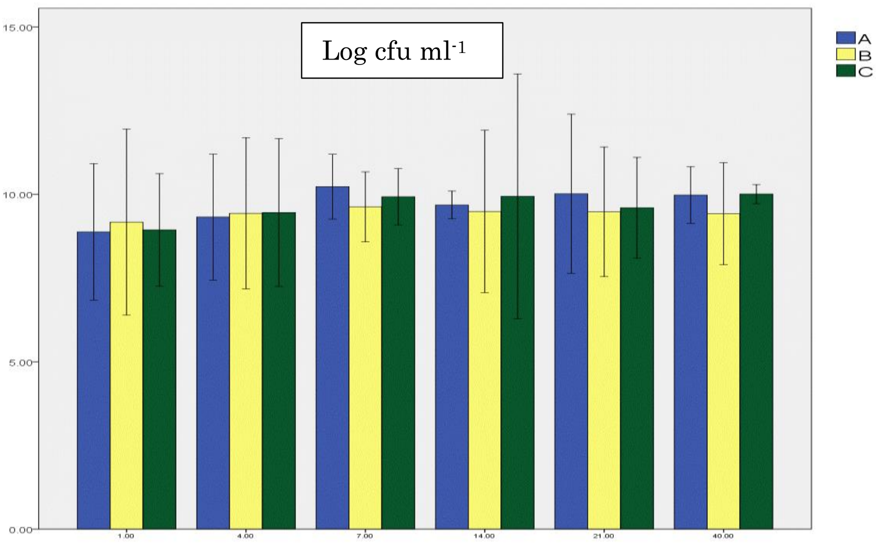
The cow milk kefir with 2% inulin exhibited the highest *Streptococci* and *Lactobacilli* counts at the end of the storage (14 days). It was explained as inulin-type fructans can promote the development of *Bifidobacteria* and *Lactobacilli* (Kef and Arslan, 2021).

The results obtained in the study was consistent with the previous reports. Witthuhn et al. (2005) observed 6.88-8.30 log cfu/ml in kefir samples, Kok-Tas et al. (2010) found 8 log cfu/ml in inulin added probiotic ayran samples. Moreover, Cetinkaya and Elal-Mus (2012) determined 4.68-8.26 log cfu/ml in 50 kefir samples from Bursa. In another study, *Lactobacillus* spp. numbers were found 9.96 log cfu/ml in kefir samples which were produced with the addition of 4% oligosaccharides (Oh et al., 2013).

Viability of *Lactococcus* spp. of kefir samples are presented in Fig 2c. The viable cell counts of *Lactococcus* spp. were 8.08-11.17 log cfu /ml during the storage. Changes in 1% inulin added kefir samples were found significant on the 4th, 7th and 14th days of storage ($p<0.05$). *Lactococcus* spp. numbers for 2% inulin added kefir samples ranged from 11.17 to 10.25 log cfu /ml on the 40th day of storage.

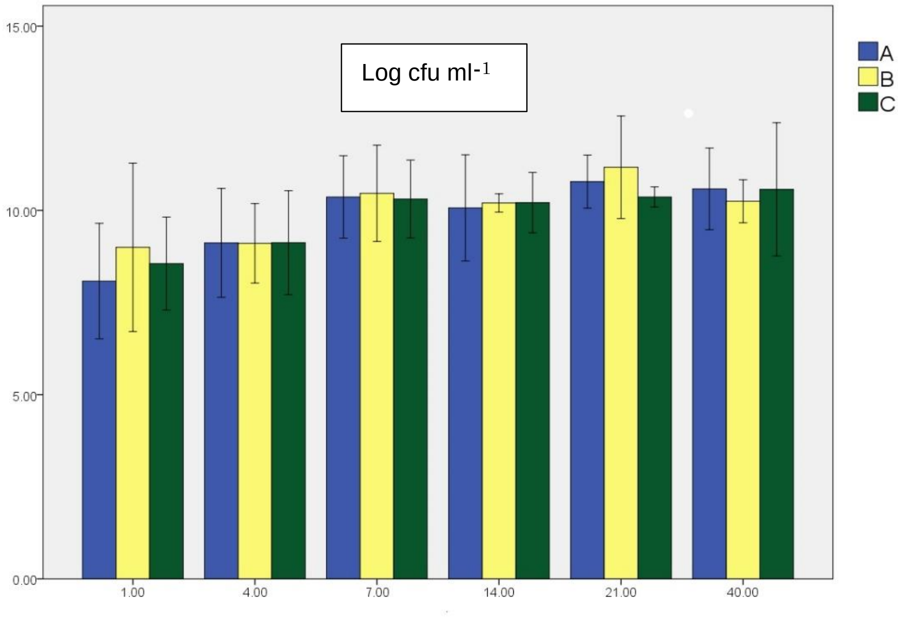


a



B

Storage days



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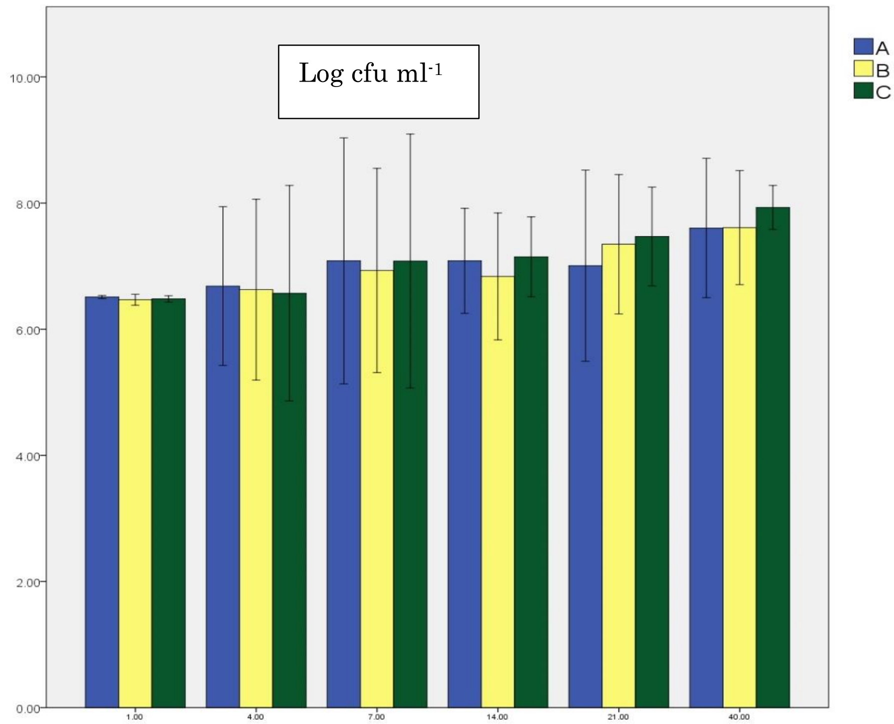
Storage days



D

Storage days

Figure 2. a) Total aerobic mesophilic bacteria numbers b) Lactobacillus spp. numbers c) Lactococcus spp. numbers d) yeast numbers (log cfu/ml) of kefir samples with the standard deviation bars. (A: 1% w/v inulin added samples; B: 2% w/v inulin added samples; C: Control groups).

Şekil 2. Kefir örneklerinin standart sapmaları ile birlikte a)Toplam aerobic mezofilik bakteri sayıları b) Lactobacillus spp. sayıları c) Lactococcus sayıları d) maya sayıları (log kob /ml) (A: % 1 inülin ilaveli örnekler; B:% 2 inülin ilaveli örnekler C: Kontrol grupları).

Garcia-Fontan et. al. (2006) found 8 log cfu/ml *Lactococcus* spp. in cows' milk kefir samples and Karatepe and Yalcin (2014) determined *Lactococcus* numbers as 7.26- 8.17 log cfu/ml in kefir samples. They also observed an increase in the viable bacteria to 8.23 log cfu/ml after 15 days of storage in their research. Kim et al. (2014) determined 8.84 log cfu /ml *Lactococcus* spp. as a dominant flora in kefir samples. The data were found similar to prior research results, but it was indicated that all kefir samples had higher bacteria numbers than the other research findings. It may be said that *Lactococcus* numbers can be affected by variables, namely inulin addition, milk type and milk's nutrient compounds, acidity of samples, and so forth.

No mould growth in all the kefir samples during the storage time was observed. However, yeast growth was significantly important in control kefir samples ($p<0.05$), but changes were not found important for inulin added samples. On the 40th day yeast numbers were higher (7.93 log cfu/ml) in control samples than the others (Fig. 2d). Other researchers determined lower numbers in yeasts counts regarded as 5.29-5.63 log cfu/ml in goat's milk kefir samples (Satir et al., 2015), 6 log cfu/ml yeasts in kefir samples after 28 day of storage (Leite et al., 2013) and 5.47, 5.44, 5.00 log cfu/ml yeasts numbers in cow's, ewe's and goat's milk kefir samples (Yaman et al., 2010).

Since the flavour of goat's milk has been found more intense in comparison to cow's milk, the production of dairy products using mixtures of goat and cow milks may be an interesting approach for the dairy market in order to add value to products, supporting some sensory and texture properties and acceptance by the consumers (Gomes et al., 2013). The sensory properties of the samples were applied by the scaling procedure. The kefir samples were evaluated for colour, texture, taste and overall acceptability (yeasty taste, fermented taste, sour taste, sour odour, viscosity, serum separation). Samples were coded with randomly chosen three numbers and served as 8°C. In the general sense, changes in acidity was found to affect the organoleptic characteristics of the products. It was found out that 1% inulin added samples preferred by the panellists took higher marks in total (4.1 points) than the others on the 40th day (Fig. 3). Ertekin and Guzel-Seydim (2009) added inulin in kefir samples in their research and they did not determine any negative effect on product quality. Tratnik et al. (2006) reported that sensorial differences were not significant in kefir samples produced with or without inulin addition until 5th or 10th days of storage, but marks given to taste of inulin added kefir samples were lower than the control samples.

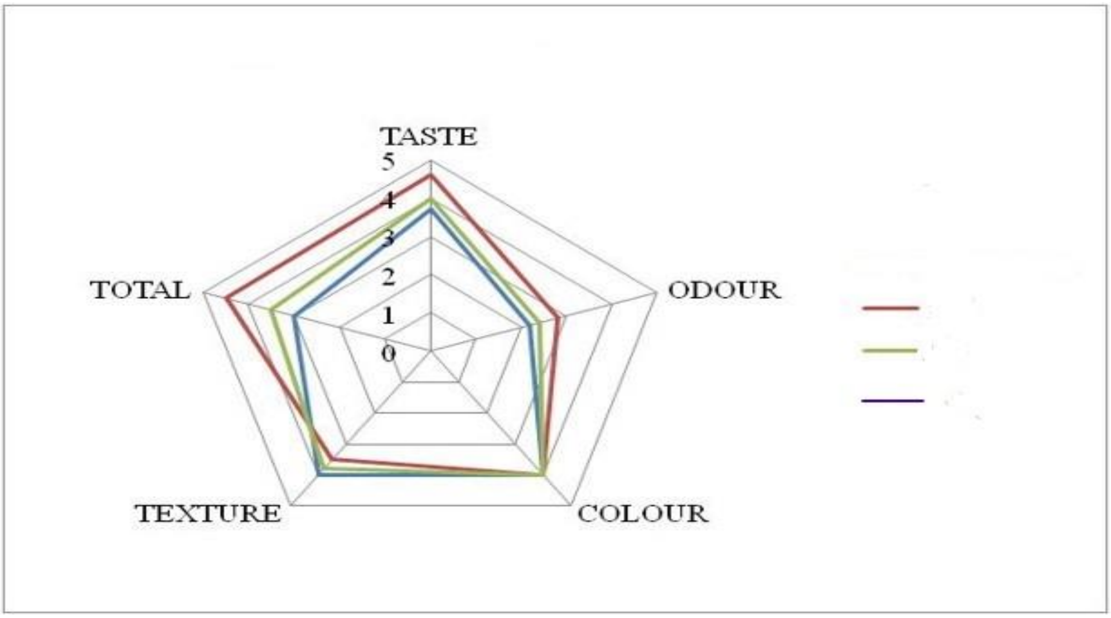


Figure 3. Sensory analyses result of kefir samples (A: 1% w/v inulin added samples; B: 2% w/v inulin added samples; C: Control groups)

Şekil 3. Kefir numunelerinin duyusal analiz sonuçları (A: %1 w/v inülin ilaveli örnekler; B: % 2 w/v inülin ilaveli örnekler; C: Kontrol grupları).

It can be concluded that inulin addition had no effect on the pH values of the product, but the lactic acid changes were found significant for inulin added samples. Inulin addition was also found out to improve viscosity, viability of *Lactococcus* spp., and *Lactobacillus* spp. and sensory properties of kefir. To this end, control kefir samples can be evaluated as a functional probiotic product because of containing >10 log cfu/ ml *Lactobacillus* spp. and *Lactococcus* spp. bacteria numbers. Fortification of goat's milk kefir with inulin can be regarded as an alternative to develop a functional beverage having health and nutritional benefits. As a prebiotic, inulin can provide viability of the probiotic bacteria in kefir for a long storage time. The sensorial properties of kefir can be enhanced with inulin addition as 1% (w/v) concentration.

Contribution of Authors

The authors declare that they have contributed equally to the article.

Conflict of Interest

Artcle authors declare that there are no conflicts of interest among them.

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