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İnkübasyon Süresinin Kefir Kültürü ile Hazırlanan Kefir İçeceklerinin Raf Ömrüne Etkisi

The Effect of Incubation Period on the Shelf Life of Kefir Beverage Prepared with Kefir Culture

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Lactococcus türleri,
Maya

Öz

Bu çalışmanın amacı farklı inkübasyon sürelerinin kefirin raf ömrüne olan etkisini incelemekti. Bu amaçla kefir örnekleri ilk önce 8, 12, 18, 24 ve 36 saat inkübasyona tabi tutularak 5 farklı kefir grubu oluşturuldu. Daha sonra 4±1°C'de 21 gün boyunca depolandı. Muhafaza süresi boyunca kefir gruplarının mikrobiyolojik (*Lactobacillus* spp., *Lactococcus* spp. ve maya), kimyasal (pH, titre edilebilir asitlik) ve duyusal analizleri yapıldı. Uzun süre inkübasyon uygulanan kefir gruplarında Laktobasil (7.80 log10 kob/mL), laktokok (7.30 log10 kob/mL) ve maya (6.14 log10 kob/mL) sayılarının daha yüksek olduğu, muhafaza sürecinde de inkübasyon süresine bağlı olarak değişimlerin olduğu görülmüştür. İnkubasyon süresi ve muhafaza süresi uzadıkça titre edilebilir asitliğin arttığı belirlenmiştir. Sonuçlar inkübasyon süresinin *Lactobacillus* spp. ve *Lactococcus* spp. sayıları, pH, asitlik üzerinde etkili olduğunu ($P<0.05$) göstermiştir.

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Abstract

The aim of this study was to examine the effect of different incubation times on the shelf life of kefir. For this purpose, kefir samples were first incubated for 8, 12, 18, 24 and 36 hours, creating 5 different kefir groups. It was then stored at 4±1°C for 21 days.. Microbiological (*Lactobacillus* spp., *Lactococcus* spp., and yeast), chemical (pH, titratable acidity), and sensory analyses of the kefir groups were performed during the storage period. The *Lactobacillus* (7.80 log10 cfu/mL), *Lactococcus* (7.30 log10 cfu/mL) and yeast (6.14 log10 cfu/mL) counts were higher in the kefir groups that were incubated for a long time, and changes were observed during the storage process depending on the incubation time. It was determined that the titratable acidity became higher as the length of the incubation and storage time increased. The results showed that the incubation time had an effect on *Lactobacillus* spp. and *Lactococcus* spp. counts, pH and acidity ($P<0.05$).

1. INTRODUCTION

The word probiotic is a Latin term that means "for life". Probiotics are foods of live microbial origin that benefit humans by maintaining the intestinal flora (FAO/WHO 2001). Probiotics ensure a stable environment for beneficial bacteria in the gastrointestinal system

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and support their function by protecting the intestinal microflora. Thus, the intestinal flora plays an active role against infections and prevents the proliferation of harmful microorganisms (Güven et al., 2021; Karahan & Güvener, 1999).

Probiotics are found naturally in fermented dairy products. One of these is kefir, which is formed as a result of ethyl alcohol and lactic acid fermentation. Kefir is a slightly acidic, refreshing fermented milk product obtained by adding kefir grains to the milk of various animals, including that of sheep, goats, camels, mares, and especially cows (Kakisu et al. 2011 Kurman et al., 1992). Kefir can be prepared from whole, semi-skimmed, or skimmed milk, as well as from vegetable sources such as rice, nuts, coconut and soy milk (Dahiya & Nigam, 2023; Gocer & Koptagel, 2023; Otles & Cagindi 2003; Rosa et al., 2017).

It has been stated that kefir was first made by the Turks in Southwest Asia (Yüksekdağ & Beyatlı, 2003). Kefir contains all the nutrients of milk as well as essential fatty and amino acids that are extremely important for the body. In addition, kefir is rich in B vitamins, vitamin K, and folic acid. Kefir is a good source of calcium and also contains potassium, iron, copper, phosphorus, magnesium, cobalt, zinc, and manganese. Kefir, which is of great importance in a healthy diet, can be consumed without problems by individuals with lactose intolerant, because it contains less lactose than milk (Saloff-Coste, 1996). During kefir formation, the metabolites produced by lactic bacteria (López-Cuellar et al., 2016, Zhang et al., 2014), strengthen the immune system by suppressing the growth of pathogenic microorganisms (Amirbozorgi et al., 2016; Erdoğmuş & Bostancı, 2020). Erdoğmuş and Bostancı (2020) reported in their study that the substances obtained from lactic acid bacteria isolated in kefir samples exhibited high antimicrobial effects on pathogenic microorganisms. In addition to its especially positive effects on the digestive system, kefir has been reported to be highly effective in controlling obesity, regulation of blood sugar, reduction in levels of serum cholesterol, regulation of blood pressure, and prevention of allergy and tumor formation (Bengoa et al., 2018; Kadıoğlu, 2017). These features are provided by kefir grains, 3 to 35mm in size and having the appearance of hard, yellowish pieces of cauliflower (Arslan, 2015). Kefir grains are composed of gelatinous colonies formed by a combination of several bacteria and yeast species. Many kinds of bacteria and yeast with symbiotic metabolic activity are effective in the formation of kefir. They ferment milk to form substances such as lactic acid, ethyl alcohol, CO_2, acetone, and acetaldehyde, diacetyl, all of which provide the organoleptic

properties of kefir (Anonymous, 2016). Furthermore, the taste and composition of kefir may vary depending on the type and characteristics of the milk used, the kefir production technique, the fermentation temperature of the milk, the time and temperature of incubation and storage conditions. The diverse microflora of the kefir culture, living or dead, are effective in the formation of the characteristic properties of kefir (Kadıoğlu, 2017). Many microorganisms form the microflora of kefir, including bacteria like *L. acidophilus, L. brevis, L. casei, Leuconostoc mesenteroides* subsp*. dextranicum, Streptococcus lactis* ssp*. cremoris, S. citrovorum, L. fermantum, L. caucasicus, L. Helveticus, Acetobacter rasens, Acetobacter aceti, S. durans, and S. diacetylactis,* with many microorganisms such as *Saccharomyces cerevisiae, Kluyveromyces marxianus* subsp. *marxianus*, and *Torulaspora delbrueckii* (Güzel-Seydim et al., 2011; Witthuhn, 2005). The types of microorganisms in the grains and their ratio to each other may vary according to the origin of the grains. In all kefir production, lactic fermentation, alcohol fermentation, kefir-specific yeast flavor formation, and slow proteolysis fermentation occur during fermentation (Konar & Şahan, 1989). In addition, the largest changes in kefir formation occur during the fermentation phase (Gawel & Gromadka, 1978). The chemical and biochemical events that began at this stage continue in the kefir storage phase (Kılıç et al., 2001). In kefir production, the fermentation time, storage time, and changes in production temperature can affect the number and type of microorganisms in the kefir and its sensory, physical, and chemical properties (Yaygın, 1995).

From this point of view, with this study, kefir groups were formed and incubated at different times to determine changes in the microflora and chemical and sensory properties of kefir samples kept for 21 days in cold storage (4±1°C) depending on the incubation times.

2. MATERIAL AND METHODS

2.1. Material

The cow milk used in kefir production was obtained from the Kafkas University Veterinary Faculty Farm, and the Lyophilised kefir culture was obtained from the Wisby company.

2.2. Preparation of Samples

Kefir production by culture; First of all, a few of the lyophilized cultures Kefir culture was prepared by making a passage. Milk with a fat-free dry matter of 9.4% and a fat content of 3.2% was pasteurized at 90°C for 5 min, cooled to 25°C, put into sterile glass bottles and inoculated with kefir culture at a rate of 4.5%. Afterwards inoculation with the culture, the milk was divided into five groups: 8 h, 12 h, 18 h, 24 h, and 36 h. Each group was incubated separately at 25±1^{o}C for the specified times. After the incubation period was completed, kefir groups were separated from the culture under sterile conditions and stored at 4°C.

2.3. Analytical Methods

To monitor the development of the kefir microflora, a 1 mL sample was taken under aseptic conditions and mixed with 9 mL of 0.1% peptone water in a sterile tube using a vortexer. Ten-fold dilutions were then prepared, taking into account the estimated number of bacteria.

For the *Lactobacillus* spp., MRS agar (Oxoid CM 361) was used. Using the spread plate method, 0.1 mL of inoculation was carried out on the medium with the pH adjusted to 5.7. After 36 h incubation at 35°C anaerobically (AnaeroGen-Oxoid), the petri dishes were evaluated. Typical colonies of 1 to3 mm in diameter were counted on the petri dishes after microscopic verification (IDF, 1983). For counting the *Lactococcus* spp., M17 Agar (Oxoid CM785) adjusted to pH 6.9 was used. After inoculating the spread plates, the samples were incubated at 35°C for 36 h aerobically. Typical colonies of 1 to 2 mm in diameter were counted after confirmation by microscopic examination (Dave & Sha, 1996). Potato dextrose agar (Oxoid CM 139) adjusted to pH 5.6 was used for counting. After inoculation and incubation at 22°C for five days, the counted colonies were evaluated. After counting the typical colonies growing in all the morphologically evaluated growth media, the amount of cfu/mL was calculated (Dave & Sha, 1996; Elmer & James, 2001). All analyses were repeated twice.

The pH analysis of the samples was carried out using a pH meter (Hanna HI 8521), and acidity in terms of lactic acid (LA)% was determined by the titration method (Meyer et al., 2007).

Sensory analysis was applied to kefir samples whose incubation period was completed. Five experienced panelists evaluated the kefir samples sensorially based on appearance, consistency, odor and taste. After the panelists had first interpreted the appearance and then

the consistency, the kefir was mixed and analyzed in terms of smell and flavor. Water was provided to refresh the mouth between samples. Each panelist evaluated the kefir according to the specified qualities using the 5-point hedonic test scale (1-worst, 5-very good) indicated on the form (Clark et al. 2008; Metin, 2006). All of the analyses were done twice.

2.4. Statistical Analysis

SPSS 18 package program was used to interpret the data obtained from the analyzes performed in duplicate. The Tukey test was used to evaluate the difference between groups. The results were presented as; mean (±) and standard error (x±Sx). (Pripp 2013).

3. RESULTS

During storage, the kefir samples showed statistically significant ($P<0.05$) changes depending on the last incubation time. In the kefir groups incubated for 8, 12, 18, 24, and 36 hours, the initial lactic acid bacteria count was determined as 5.17, 5.17, 7.64, 7.10, and 7.80 log cfu/g, respectively. On day 21 of cold storage, the lactic acid bacteria count of the kefir samples (incubated for 8, 12, 18, 24, and 36 hours) was determined to be 5.15, 5.15, 7.55, 4.95 and 7.76 log cfu/g, respectively. While Lactococcus bacteria counts in the groups were 4.80-7.30 log10 cfu/ml at the beginning, they changed between 4.46-7.76 log10 cfu/ml at the end of the 21st day. At the end of the 21-day storage period, the lowest yeast count was 3.42 log10 cfu/ml (8 h.) and the highest yeast count was 5.15 log10 cfu/ml (36 h.) ($P<0.05$). The difference between the kefir groups was statistically significant. The changes in the kefir groups (0, 1, 3, 7, 10, 14, 18, and 21 days) are given in Table 1.

At the end of the incubation, the highest pH value (5.10) was in the kefir group incubated for 8 h, and the lowest (4.40) was in the kefir group incubated for 36 h. This did not change during the cold storage period. At the end of the 21-day storage period, the acidity of the groups varied between 0.42-0.69%. The changes in the kefir groups (0, 1, 3, 7, 10, 14, 18, and 21 days) are given in Table 2. After the sensory analyses, the group with the highest score from the panelists was the kefir group incubated for 18 hours. The results of the sensory analysis are given in Table 3. and Figure 1.

Table 1. Average values for the microbiological parameters of the samples during the storage period (log10 cfu/mL±Std deviation)

	Groups	0. day (X±Sx)		1. day (X±Sx)		3. day (X±Sx)		7. day (X±Sx)		10. day (X±Sx)		14. day (X±Sx)		18. day (X±Sx)		21. day (X±Sx)		P
	Culture	5.10	0.02^{BCa}	5.10	0.02^{BCa}	5.11	0.04^{Ca}	5.12	0.03^{Ca}	5.12	0.02^{Ca}	5.10	0.02^{BCa}	4.98	0.02^{Cb}	4.95	0.03^{Cb}	*
	8. h.	5.17	0.04^{b}	5.16	0.04^{b}	5.17	0.03^{b}	5.16	0.02^{b}	5.16	0.03^{b}	5.16	0.03^{b}	5.15	0.03^{b}	5.15	0.02^{b}	-
Anaerobic	12. h.	5.17	0.02^{b}	5.17	0.02^{b}	5.16	0.04^{b}	5.16	0.04^{b}	6.17	0.02^{b}	5.17	0.02^{b}	5.16	0.04^{b}	5.15	0.02^{b}	-
Lactobacillus spp.	18. h.	7.64	0.02^{Ba}	7.62	0.03^{Ba}	7.62	0.03^{Ba}	7.60	0.02^{Ba}	7.58	0.04^{Aa}	7.58	0.03^{Aa}	7.58	0.02^{Aa}	7.55	0.02^{Aa}	-
	24. h.	7.10	0.02^{BCa}	5.10	0.02^{BCb}	5.11	0.04^{Cb}	5.12	0.03^{Cb}	5.12	0.02^{Cb}	5.10	0.02^{BCb}	4.98	0.02^{Cc}	4.95	0.03^{Cc}	*
	36. h.	7.80	0.04^{a}	7.82	0.03^{a}	7.81	0.02^{a}	7.85	0.02^{a}	7.83	0.03^{a}	7.81	0.02^{a}	7.80	0.02^{a}	7.76	0.02^{a}	-
	p		*		*		*		*		*		*		*		*	
	Culture	4.60	0.04^{Dd}	4.60	0.03^{Dd}	4.60	0.02^{Dd}	4.58	0.02^{Dd}	4.55	0.03^{Dd}	4.53	0.02^{Dd}	4.49	0.04^{Cd}	4.46	0.02^{Cd}	-
	8. h.	5.12	0.03^{b}	5.12	0.02^{b}	5.11	0.04^{b}	5.12	0.02^{b}	5.12	0.02^{b}	5.12	0.03^{b}	5.11	0.02^{b}	5.11	0.04^{b}	-
	12. h.	4.80	0.03^{Dc}	4.80	0.02^{Dc}	4.85	0.03^{Dc}	4.84	0.02^{Cc}	4.86	0.02^{Cc}	4.82	0.04^{c}	4.80	0.02^{Bc}	4.76	0.03^{Bc}	-
Lactococcus spp.	18. h.	5.11	0.02^{Cb}	5.11	0.03^{Cb}	5.11	0.02^{Cb}	5.11	0.04^{Cb}	5.11	0.02^{Cb}	5.11	0.03^{Cb}	5.10	0.02^{Bb}	4.98	0.02^{Bc}	-
	24. h.	5.18	0.03^{Cb}	5.18	0.02^{Cb}	5.18	0.03^{Cb}	5.18	0.02^{Cb}	5.17	0.04^{Cb}	5.17	0.02^{Cb}	5.17	0.03^{Bb}	5.16	0.02^{Bb}	-
	36. h.	7.30	0.02^{Ba}	7.33	0.03^{Ba}	7.35	0.02^{Ba}	7.34	0.03^{Ba}	7.38	0.02^{Ba}	7.32	0.03^{Ba}	7.29	0.03^{Aa}	7.26	0.04^{Aa}	-
	p		*		*		*		*		*		*		*		*	
	Culture	6.50	0.03^{Aa}	6.48	0.02^{Aa}	6.48	0.02^{Aa}	6.45	0.02^{Aa}	6.40	0.02^{Aa}	6.38	0.04^{Aa}	6.37	0.02^{Aa}	6.35	0.03^{Aa}	-
	8. h.	3.50	0.02^{Cd}	3.50	0.03^{Cd}	3.51	0.02^{Cd}	3.53	0.02^{Cd}	3.51	0.03^{Cd}	3.49	0.02^{Cd}	3.47	0.03^{Bd}	3.42	0.02^{Cd}	-
	12. h.	3.50	0.02^{Cd}	3.51	0.02^{Cd}	3.53	0.04^{Cd}	3.55	0.02^{Cd}	3.57	0.02^{Cd}	3.60	0.02^{Cd}	3.62	0.02^{Cd}	3.65	0.03^{Cd}	-
Yeast	18. h.	4.90	0.04^{Bc}	4.90	0.02^{Bc}	4.92	0.02^{Bc}	4.95	0.03^{Bc}	4.93	0.03^{Bc}	4.95	0.03^{Bc}	4.93	0.03^{Bc}	4.94	0.03^{Ac}	-
	24. h.	4.80	0.02^{Bc}	4.80	0.02^{Bc}	4.82	0.02^{Bc}	4.84	0.03^{Bc}	4.86	0.02^{Bc}	4.87	0.02^{Bc}	4.85	0.02^{Bc}	4.89	0.02^{Bc}	-
	36. h.	6.14	0.02^{Ab}	6.14	0.04^{Ab}	6.15	0.02^{Ab}	6.14	0.02^{Ab}	6.15	0.03^{Ab}	6.16	0.04^{Ab}	5.15	0.02^{Ac}	5.15	0.02^{Ac}	*
			*		*		*		*		*		*		*		*	

Capital letters (A, B, C,...) indicate statistical difference between groups in the same column, while miniscule letters (a, b, c,..) indicate the statistical difference between groups on the same line. *: The statistical difference is important (P<0.05).

Table 2: Average values for the chemical parameters of samples during the storage period (log10 cfu/mL±Std deviation)

	Groups	0. day (X±Sx)		1. day (X±Sx)		3.day (X±Sx)		7.day (X±Sx)		10.day (X±Sx)		14.day (X±Sx)		18.day (X±Sx)		21.day (X±Sx)		P
pH	8. h.	6.20	0.03^{Aa}	6.10	0.02^{Aa}	6.00	0.02^{Ab}	6.00	0.03^{Ab}	5.80	0.02^{Ac}	5.60	0.02^{Ad}	5.20	0.02^{Ae}	5.10	0.02^{Ae}	*
	12. h.	6.10	0.02^{Aa}	6.00	0.02^{Aa}	6.00	0.02^{Aa}	5.90	0.02^{Ab}	5.70	0.02^{Ac}	5.30	0.02^{Bd}	5.30	0.02^{Ad}	5.00	0.02^{Ae}	*
	18. h.	6.05	0.02^{Aa}	5.80	0.04^{Bb}	5.30	0.03^{Bc}	5.20	0.02^{Bc}	5.10	0.03^{Bc}	4.90	0.03^{Bd}	4.80	0.03^{Bd}	4.70	0.02^{Be}	*
	24. h.	6.00	0.02^{Aa}	5.60	0.02^{Bb}	5.30	0.02^{Bc}	5.20	0.02^{Bc}	5.00	0.02^{Bd}	4.90	0.02^{Bd}	4.80	0.02^{Bd}	4.60	0.03^{Be}	*
	36. h.	5.80	0.04^{Ba}	5.30	0.03^{Cb}	5.10	0.02^{Cb}	5.00	0.02^{Cc}	4.80	0.03^{Cd}	4.70	0.02^{Cd}	4.60	0.02^{Ce}	4.40	0.02^{Cf}	*
	p		*		*		*		*		*		*		*		*	
	8. h.	0.21	0.01^{Cg}	0.23	0.00^{Df}	0.30	0.00^{Ce}	0.31	0.00^{Bd}	0.33	0.00^{Cd}	0.38	0.01^{Cc}	0.40	0.00^{Bb}	0.42	0.01^{Ba}	*
	12. h.	0.31	0.01^{Be}	0.35	0.00^{Cc}	0.32	0.00^{Bd}	0.35	0.00^{Bc}	0.39	0.01^{Cb}	0.40	0.01^{Bb}	0.42	0.00^{Ba}	0.45	0.01^{Ba}	*
	18. h.	0.37	0.00^{Bd}	0.42	0.01^{Cd}	0.50	0.01^{Ac}	0.55	0.01^{Ac}	0.58	0.00^{Bb}	0.61	0.00^{Ab}	0.65	0.00^{Aa}	0.66	0.00^{Aa}	*
Acidity	24. h.	0.43	0.01^{Ad}	0.49	0.00^{Bc}	0.52	0.00^{Ac}	0.57	0.00^{Ab}	0.59	0.01^{Bb}	0.62	0.00^{Ab}	0.66	0.01^{Aa}	0.68	0.00^{Aa}	*
	36. h.	0.50	0.00^{Ad}	0.53	0.01^{Ac}	0.55	0.00^{Ac}	0.59	0.01^{Ab}	0.60	0.01^{Ab}	0.63	0.00^{Ab}	0.67	0.01^{Aa}	0.69	0.00^{Aa}	*
	p		*		*		*		*		*		*		*		*	

Capital letters (A, B, C,…) indicate statistical difference between groups in the same column, while miniscule letters (a, b, c,..) indicate the statistical difference between groups on the same line. *: The statistical difference is important (P<0.05).

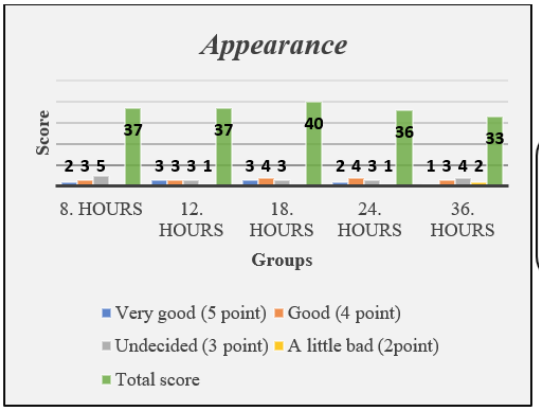

Figure 1. Appearance analysis results of kefir samples

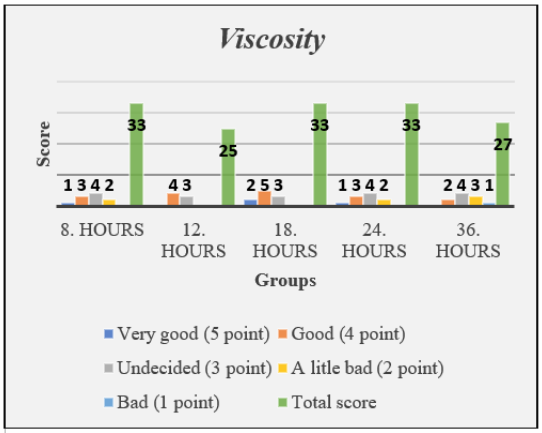

Figure 2. Viscosity analysis results of kefir samples

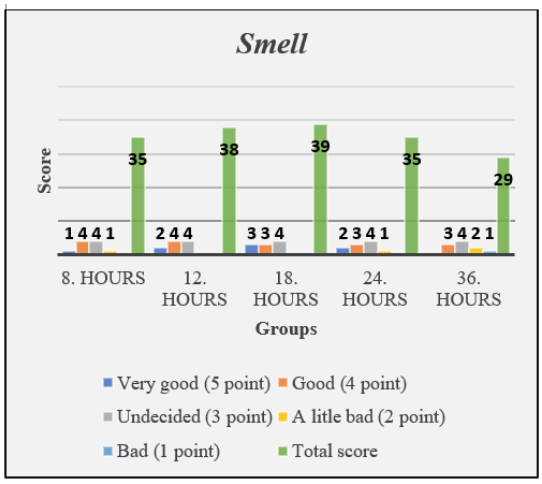

Figure 3. Smell analysis results of kefir samples

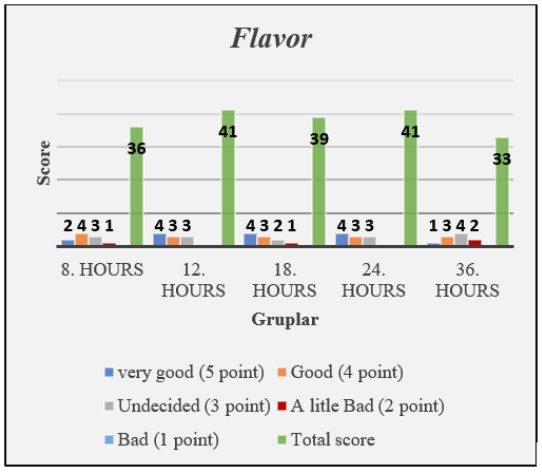

Figure 4. Flavor analysis results of kefir samples

4. DISCUSSION

The yeast and bacteria in the microflora of kefir culture are directly effective in the formation of the microflora of the kefir without any heat treatment. Accordingly, the bacterial load in kefir may vary depending on the microflora characteristics of the kefir culture used. The location of the yeasts in the kefir grain and their ability to ferment lactose are properties that can directly affect the microflora. These active flora have been reported to actively maintain their vitality in the final product (Farnworth, 2005). In this study, the kefir culture retained its vitality during storage and, during the storage process, depending on the incubation period, changes occurred in the kefir groups prepared using the kefir culture.

The bacterial load and microflora of the kefir samples varied depending on the incubation time. It was observed that the number of lactic acid bacteria and yeasts forming the kefir microflora increased significantly as the incubation period increased.

The initial lactobacillus counts were found to be quite high in the kefir groups with an incubation period of 8 and 36 h. At the end of the storage period, the highest lactobacillus count was also in parallel with these groups. This may have been due to the fact that increased acidity is well-tolerated in lactobacilli.

Güzel-Seydim et al. (2000) stated that lactic acid bacteria (LAB) increased during the fermentation period because of the increase in the amount of lactic acid. Similarly, in this study, it was observed that with the increase of acidity, the LAB increased throughout the storage period. During the storage period, the results showed statistically significant changes in proportion to the final number of fermentation hours. During the trial period, the lactobacilli counts in the kefir groups were between 4.95 and 7.85 log10 cfu/mL, which were similar to those reported by Kesmen and Kaçmaz (2011) but lower than the values of Garrote et al. (2001).

Güzel-Seydim et al. (2005) stated in their study that at 0, 5, 10, 15, and 22 h of kefir fermentation, the LAB numbers gradually increased compared to the initial numbers. In this study, it was observed that the LAB numbers increased in parallel with the fermentation period. Throughout the cold storage duration, the bacterial load in the kefir groups changed in proportion to the bacterial load at the end of the incubation period, and the lactobacilli count decreased, similar to the findings of Iriyogen et al. [35]. This decrease in kefir culture was statistically significant in kefir groups incubated for 24 hours. ($P<0.05$).

Fontan et al. (2006). stated that lactococci were dominant in the microbial flora in the first 48 h of fermentation, but that the number of lactobacilli surpassed them after 48 h. In this study, lactobacillus counts were more dominant in the first hours of fermentation (5.1 log cfu/mL), whereas after 36 h, lactococci were more dominant (7.3 log cfu/mL), with numbers increasing in parallel with the increase in fermentation time and approaching the lactobacillus counts. The study of Kök-Taş et al. (2012) reported the lowest number of *Lactococcus* spp. as 6.3 log cfu/mL, and the highest as 9.1 log cfu/mL, whereas the lactococcus counts in the kefir groups in this study during the trial period exhibited lower values of between 4.46 and 7.38 log cfu/mL. This difference may be due to the microflora variations in the kefir cultures used or differences in the production technique.

Irigoyen et al. (2005). and Öner et al. (2010). stated in their studies that there was a reduction in the number of lactococcus bacteria during the cold storage period. Similarly, in this study, compared to the initial numbers, there was a decrease in the number of lactococci in the kefir groups on the 21st day of cold storage. Statistically significant decreases were seen in the culture during storage. The 12- and 36-h kefir incubation groups were found to have 4.76, and 7.26 log cfu/mL at the end of the 21st day, respectively.

The Turkish Food Codex Fermented Milks Communiqué states that kefir must contain yeast at a level of at least 10^4 cfu/mL (Anonymous, 2009). The results obtained from this study were determined to be in accordance with the Turkish Food Codex Communiqué on Fermented Milk. Although the amount of yeast in the samples decreased compared to the kefir culture at the end of the first 8 h of incubation, it increased in parallel with the increase in the incubation period, as in the study of Güzel-Seydim (2005)., and approached the number in the kefir culture at the end of the last 36 h of the incubation period. As in the studies of Güzel-Seydim et al. (2005) and Öner et al. (2010), during the storage period, an increase in the kefir cultures was seen in the kefir groups incubated for 12, 18, and 24 h. However, a statistically significant decrease in kefir cultures was observed in the kefir groups incubated for 36 h ($P<0.05$). During the trial period, a change varying between 3.420 and 6.500 log cfu/mL was found which was lower than in the study of Karagözlü (1990).

As the incubation time of kefir samples increased, the pH value decreased. In the study of Graciela et al. (2001), the pH value of kefir samples was measured between 3.5 and 4. Another study found pH values of kefir groups to be 4.08-4.10 at the 24th h of the storage period (Sezer, 2003), whereas Ergin et al. (2017) reported kefir pH values of between 4.54 and

4.59. In this study, the pH values of the kefir groups were determined to be 4.40-5.10 at the end of the 24th h. In a study in which pH analysis was performed on kefir samples incubated for 24 h, pH changes were between 6.60 and 3.79 during the storage period (Tan & Ertekin, 2017). In this study, pH values of the kefir groups incubated for 24 h were between 6.00 and 4.60. It was thought that the pH differences determined in the kefir samples might have occurred because of the chemical properties of the milk used in the study or the microflora of the kefir culture.

As in the studies of Sezer (2003) and Karagözlü (1990) the pH values of the kefir groups decreased and acidity increased during the storage period. This may be associated with the decrease in pH as a result of the bacteria of the lactobacillus group increasing the production of lactic acid in the environment. Ergin et al. (2017) stated that the acidity value of their samples varied between 0.73% and 0.87% during storage, whereas the acidity of the kefir samples in this study during storage was between 0.21% and 0.69%. In a study, different types of kefir were produced and the acidity value of plain kefir was determined as 0.300% after 12 hours of incubation (Harmankaya, et al. 2019). Similarly in this study, upon chemical analysis of the kefir groups, acidity values were the similary (0.31% LA) at the end of the 12^{th} hour incubations. Among all groups, at the end of the storage period, the kefir group incubated for the longest time (36 h) showed the highest acidity (0.69% LA). At the end of the study, it was concluded that change in the duration of the incubation affected the acidity and the acidity increased as the storage time was extended ($P<0.05$).

In a number of studies it is stated that changes in the chemical and microbiological properties of kefir directly affect its sensory properties (Tekinşen & Atasever, 1994; Toklu, 1999). In this study, it was observed that chemical and microbiological changes that occurred depending on the incubation period also affected the sensory properties.

Sensually, good kefir should have a fluid consistency, a homogeneous and light appearance, and a light yeasty taste. The aroma should be experienced when consumed, and it should have a refreshing quality (İnal, 1990). In the sensory analysis of the kefir evaluated considering these properties, the kefir group incubated for 18 h received the highest score in appearance (40). The highest score for viscosity was found for the kefir incubated for 8 h, 18 h, 24 h and the lowest for that incubated for 36 h. The panelists stated that the consistency of the kefir increased as the duration of incubation increased and it became unacceptable by the end of 36 h. In the smell analysis, the kefir group that was least liked was the one that had

been incubated for 36 h. This was thought to have been the result of the increase in acidity. In the flavor analysis, the 12th (41) and 24th (41) h incubation groups had the highest scores. This may have been because of the amounts of flavor-affecting substances such as CO_2, lactic acid, ethanol, acetone, and acetaldehyde formed during the fermentation period (Vedamutlu, 1997).

5. CONCLUSION

In conclusion, it was observed that the incubation period was effective on the acidity and pH values of the kefir, especially for the long storage period. The acidity of the kefir groups increased with the long incubation period and the kefir was not acceptable in terms of sensory aspects. Although the lactobacillus counts exhibited a decrease in the kefir groups incubated for 12, 18, and 24 h, they increased with rising titratable acidity at the end of the 36th h. At the end of the study, the highest lactobacillus counts were found in the kefir groups incubated for 8 and 36 h. This can be explained by the ability of lactobacilli to tolerate high acidity well. Similarly, at the end of the study, lactococcal counts were found to be the highest in kefir incubated for 36 h. By resisting the developing acidity as the kefir incubation period increased, the yeast reached the highest counts in the kefir groups that were incubated for the longest time. At the end of the study, it was concluded that the incubation period had affected the chemical, microbiological, and sensory properties of the kefir.

Conflict Of Interest

The article authors declare that there is no conflict of interest between them.

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