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INVESTIGATION OF THE USABILITY OF COMMERCIAL KEFIR BEVERAGES AS INOCULUM IN HOMEMADE KEFIR PRODUCTION

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ABSTRACT
This study examined the potential usage of commercial kefir beverages in producing homemade kefir beverages. For this purpose, two different commercial kefir beverages were used in the fermentation. Microbial growth kinetics, viscosity and pH were monitored during the fermentation process of kefir samples. The logistic model was fitted to lactic acid bacteria, lactococci, total mesophilic aerobic bacteria (TMAB), pH and viscosity growth kinetics. The generation study was completed by repeatedly passing the kefir produced for four days. *Lactic acid bacteria*, *Lactococci*, and TMAB counts remained stable in the following generations. Lactobacilli counts decreased in both brands, while TMAB and lactococci decreased in brand A during storage. Syneresis values under storage conditions were high for both brands. This study shows that kefir can be produced at home using commercial kefir beverage brands and can meet the requirements of Codex Alimentarius if the necessary hygiene conditions and incubation temperature are provided, but structural stability during storage is weak.
Keywords: Kefir, storage, fermentation, commercial kefir, traditional production

TİCARİ KEFİR İÇECEKLERİNİN EV YAPIMI KEFİR ÜRETİMİNDE İNOKULUM KAYNAĞI OLARAK KULLANILABİLİRLİĞİNİN ARAŞTIRILMASI

ÖZ
Bu çalışma, ticari kefir içeceklerinin ev yapımı kefir içecekleri üretiminde potansiyel kullanımını incelemiştir. Bu amaçla, fermantasyonda iki farklı ticari kefir içeceği kullanılmıştır. Kefir örneklerinin fermantasyon süreci boyunca mikrobiyal büyüme kinetiği, viskozite ve pH izlenmiştir. Laktik asit bakterileri, laktokoklar, toplam mezofilik aerobik bakteriler (TMAB), pH ve viskozite değişiminde lojistik model uygun bulunmuştur. Üretilen kefir dört gün boyunca rejenere edilerek üretim çalışması tamamlanmıştır. Laktik asit bakterileri, Lactococci ve TMAB sayıları sonraki nesillerde sabit kalmıştır. Depolama boyunca Laktobasil sayısı her iki markada da azalırken, TMAB ve laktokoklar depolama sırasında A markasında azalmıştır. Depolama koşullarında sinerisis değerleri her iki marka için yüksek

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bulunmuştur. Bu çalışma, kefirin ticari kefir içecek markaları kullanılarak evde üretilebileceğini ve gerekli hijyen koşulları ve inkübasyon sıcaklığı sağlandığı takdirde Codex Alimentarius gerekliliklerini karşılayabileceğini ancak yapısal stabilitenin zayıf olduğunu göstermektedir.
Anahtar kelimeler: Kefir, depolama, fermantasyon, ticari kefir, geleneksel üretim

INTRODUCTION

Kefir is a fermented beverage originating in Eastern Europe, the Balkans, and the Caucasus. It has a sour, viscous, and slightly alcoholic flavor. (Sharifi et al., 2017). Studies have shown that kefir consumption has a high potential to balance the gut microbiota, reduce diarrhea and constipation, help improve intestinal permeability and regulate the immune system (Prado et al., 2015). There has been a growing interest in kefir consumption due to its potential health benefits and unique flavor.

There are two methods for making kefir: industrial and traditional/homemade. Traditional production is made from kefir grains, which include polysaccharide kefiran and pentasaccharide kefirose, which hold the microbiota (Guzel-Seydim et al., 2021; Kök-Taş et al., 2013). The microbial composition of kefir grains may vary (Gul et al., 2015; Satir and Guzel-Seydim, 2016). Kefir beverages may show different structural, aromatic, and sensory characteristics due to the variable microbiota of kefir grain, the origin of kefir grains, the type of milk, incubation temperature and duration, storage and sanitation conditions. Especially in cases where the kefir grain is not stored under hygienic conditions or its activity is reduced, significant unpleasant differences in the taste, aroma, and texture characteristics of kefir beverages occur (Kim et al., 2018). Besides the short shelf life, kefir grains have a complex structure that is unsuitable for the commercial production of a standard product (Nejati et al., 2020).

The manufacturing of kefir involves the use of microorganisms isolated from kefir grains or starter cultures that comprise freeze-dried lactic acid bacteria and yeasts (Gul et al., 2018; Wang et al., 2021). Starter cultures are available in liquid, lyophilized, and frozen forms. The most commonly used starter culture is lyophilised starter culture (DVS, direct-vat-set) and kefir starter cultures produced with these techniques are preferred in industrial production (Prado et al., 2015).

Making fermented products in homes is quite widespread. Some claimed that they could not produce a product with the desired qualities when they employed commercial products as the inoculum during production. The problems are mainly weak viscosity and insufficient flavor formation. This research investigates the feasibility of using commercial kefirs as a source of inoculum in kefir production when the required hygienic and temperature conditions are provided. This study also aimed to develop an alternative method to home production with kefir grains to standardize the taste, aroma and texture of kefirs produced using commercial kefir drinks as starter culture.

Kim et al. (2018) applied the backslopping fermentation method for producing a kefir drink, using traditional kefir produced with kefir grains as the stock culture. The authors stated that the backslopping method is feasible to scale up the production of kefir beverage and backslopped kefir contains the original kefir microbiota with a decreased yeast population. Alves et al. (2023) produced kefir beverages in household conditions with kefir grains. The authors stated that kefir produced using UHT milk under household conditions can provide the Codex Alimentarius requirements. Beverages maintain their properties about physicochemical composition after fermentation and refrigerated storage.

To the best of our knowledge, this study will be the first to investigate the potential use of commercial kefir drinks for fermentation. The two most preferred brands of kefir beverages were used, and the quality parameters of kefir samples were evaluated regarding microbial, rheological, sensory, and physicochemical properties. Fermentation, generation, and storage conditions were also investigated deeply for the first time in this type of production.

MATERIALS AND METHODS

Materials

The two preferred commercial plain kefir beverages and full-fat UHT milk were purchased from a chain store in Bolu City. Purchased products are coded as Brand A and Brand B. Brand A kefir contains 3.2g /100mL of fat, 3.2g/100mL of carbohydrate, and 2.7g/100mL of protein. Brand B kefir includes 2.4g/100mL of carbohydrates, 2g/100mL of fat, and 2.7g/100mL of protein. MRS Agar, M17 Agar, YGC Agar, and PCA Agar were purchased from Merck (Darmstadt, Germany).

Kefir production

Kefir production was carried out with commercial kefir drinks. In preliminary studies, different inoculation rates (1, 3, and 5%) were tested at 25 °C. Preliminary studies showed that 1% and 3% inoculation rates were insufficient for fermentation. The incubation time was prolonged to 30 and 28 h, respectively. However, we achieved a 24-hour incubation by using 5% inoculation rates. The inoculation rate of 5% was chosen as a suitable rate for acidification. After inoculating 5% of two brands of kefir beverages into 2L UHT milk, the samples were kept at 25 °C for about 24 hours. The kefir samples were filled into sterile bottles and stored at 4 °C under refrigerator conditions for 28 days. Produced kefir samples are coded as Brand A and Brand B. On the labels of kefirs supplied from Brand A, it was declared that kefirs were made from homogenized and pasteurized milk and the starter culture content included kefir yeasts, kefir culture and probiotic microorganisms (*Bifidobacterium, Lactobacillus Acidophilus*). Similarly, homogenized and pasteurized milk was used in Brand B, and the starter culture content was declared similar to that of Brand A. The Brand A microbial counts were 7.68±0.03, 8.32±0.03, 1.36±0.01 and 6.90±0.08 log CFU/ mL and Brand B were 7.62±0.04, 8.04±0.02, 4.28±0.11 and 6.53±0.03 log CFU/mL for *Lactobacilli, Lactococci*, yeast and TAMB, respectively. The production of kefir beverages with commercial kefir beverages was conducted twice.

Monitoring the fermentation process

Microbial growth kinetics, viscosity and pH of kefir samples were monitored throughout fermentation. The pH was recorded every hour of fermentation. For microbial growth kinetic analysis, microbial counting was performed every 5 hours during fermentation. The acidification rate (V_{max}) was calculated by the pH change (dpH/dt) with time. T_{max} (h) is the time when V_{max} reached the end of incubation; $tpH_{4.5}$ was the time to reach pH 4.5; t_f (h) was the time of completion of fermentation (Atalar, 2019).

Microbiological analysis

The microbiological analysis described below was performed for all kefir samples during fermentation, generation, and storage. Serial dilutions of kefir in 0.1% peptone water were used to investigate the development of microflora. De Man, Rogasa, and Sharp (MRS) agar medium were used to determine the number of lactobacilli in kefir samples. Analyses were performed by pour plate culture counting and incubation was carried out under anaerobic conditions (GasPak™) at 30 °C for 48 hours (Satir & Guzel-Seydim, 2015). M17 agar medium was incubated at 30 °C for 48 h to determine lactococci bacterial counts (Grønnevik et al., 2011). Yeast Extract Glucose Chloramphenicol (YGC) agar medium was used to determine the yeasts in kefir samples. The analysis was carried out using spreading plate method. Petri dishes were incubated at 25 °C for 3 days under aerobic conditions (Teijeiro et al.,2018). Plate Count Agar (PCA) was used to count the total mesophilic aerobic bacteria (TMAB) in kefir samples. The spread plate method was used and samples were incubated under aerobic conditions at 30 °C for 2 days (Corona et al., 2016).

Viscosity analysis

Kefir samples were analyzed in triplicate at 15 °C using a viscometer (AND vibro viscometer SV-10, Japan). Each measurement was performed 9 times (0, 15, 30, 45, 60, 75, 90, 105 and 120 seconds) for 2 minutes at 15-second intervals. These measurements were averaged and expressed in mPa.s (Sarica and Coşkun, 2020).

Generation study
A generation study was performed to investigate the possibility of kefir production using commercial kefir samples many times. The generation study was carried out by the method of Aydemir (2020). The first generation was done with the first kefir beverage produced with commercial kefir beverages. The next kefir production was carried out using a 24-hour kefir sample. In this way, 4 generations of yogurt production were carried out in succession. Four passages were made from kefir produced by using commercial kefir beverages.

Storage analysis
The kefir drinks produced with commercial kefir samples were stored under refrigerator conditions (4 °C) for 4 weeks, on day 1, day 7, day 14, day 21, and day 28. Syneresis, titratable acidity and pH, color analysis, microbiological and sensory analysis were performed during storage.

Syneresis
Kefir samples (25 g) were weighed into a centrifuge tube and centrifuged at 1250 g for 10 min at 4 °C (Sigma 2-16 KC, Germany). The supernatant was poured off and the collected kefir was weighed (final weighing) (Sodini et al., 2005). Serum separation was calculated by means of the following formula (Eq.1)

$$\text{Syneresis (\%)} = \frac{\text{final weighing–tare}}{\text{amounts of sample}}\ x\ 100 \qquad \text{(Eq.1)}$$

Titratable acidity and pH
The titrimetric method determined the titration acidity of kefir samples as % lactic acid. A digital pH meter was used to measure the pH of the samples. (Orion Star A211, Waltham, MA, USA).

Color analysis
The color parameters of kefir samples were measured with a CIE (International Commission on Illumination) Minolta CR-400 (Osaka, Japan) color analyzer. Before the measurement, the device was calibrated against the white plate. Samples were placed in the glass sample cup of the device and the L*, a*, and b* values of the samples were read. L*; brightness, a*; red-greenness, b*; means yellow-blue.

Sensory analysis
The evaluation was performed using the nine-point hedonic scale. Kefir beverages were analyzed by 15 trained panelists consisting of food engineering department members and graduate students on days 1, 7, 14, 21 and 28 of storage.

Statistical analysis
Multiple comparison tests determined the differences between the kefir drinks. The normality was determined by the Shapiro-Wilk test. One-way ANOVA and Tukey post hoc tests were used to determine the differences when the data were distributed normally. If the data were non-parametric, the Kruskal Wallis test and Mann Whitney U pairwise comparison tests were performed. SPSS 23.0 (IBM, SPSS Statistics 23) program was used for statistical analyses. All analyses were carried out in triplicate for each duplicate sample.

RESULTS AND DISCUSSION

Monitoring the fermentation process

Microbial growth kinetic

For microbial growth kinetic analysis, *lactobacilli*, *lactococci*, total mesophilic aerobic bacteria and yeast were counted every 5 hours during fermentation. The changes in *lactobacilli* count during the incubation of kefir samples obtained using commercial kefir drinks as starter cultures are shown in Figure 1. The provided graph illustrates the growth of *lactobacilli* overtime during the fermentation process for two different brands (Brand A and Brand B). Both brands exhibit a typical logistic growth curve, which is common in microbial growth. The logistic model fits the data well, suggesting that bacterial growth follows a typical S-shaped curve. This indicates that initially, bacteria grow rapidly, but as resources become limited, the growth rate slows down and eventually reaches a plateau.

In terms of growth rate, Brand A shows a faster growth rate for *lactobacilli* than Brand B, especially between 10 and 15 hours. Brand A kefir samples fitted the logistic model better than brand B samples, with adjusted R^2 values of 0.9602 and 0.8779 for brand A and B kefir samples, respectively. In brand A kefir samples, the

number of lactobacilli, 6.30 log CFU/ mL at the beginning of fermentation, was determined as 7.28 log CFU/mL at the end of the incubation period. The increase in *lactobacilli* counts was significant up to the 15th hour ($P<0.05$), and the increase was not significant after this period. In brand B, *lactobacilli* were 6.40 log CFU/ mL after inoculation and 7.42 log CFU/ mL at the end of incubation. Unlike brand A, the increase in the number of *lactobacilli* is significant until the 20th hour. ($P<0.05$). Similar findings were reported in the literature. Traditional and back-sloping methods for kefir fermentation were compared, and lactic acid bacteria increased between 7 and 9 log CFU/mL during fermentation in both kefir types (Kim et al., 2018). In kefir samples produced with 2% kefir grains, *lactobacilli* count increased to 5.33 log CFU/mL within 12 hours. At the end of the fermentation process, it was reported as 7.63 log CFU/mL (Hikmetoglu et al., 2020). In kefir samples made with 10% kefir grains under home conditions, the LAB count reached 7x10^{7} CFU/mL. (Alves et al., 2021). The number of *lactobacilli* after fermentation in kefir samples made with 3% bacterial strain and 2% yeast kefir grains was reported as 8 log CFU/mL (Abdolmaleki et al., 2015).

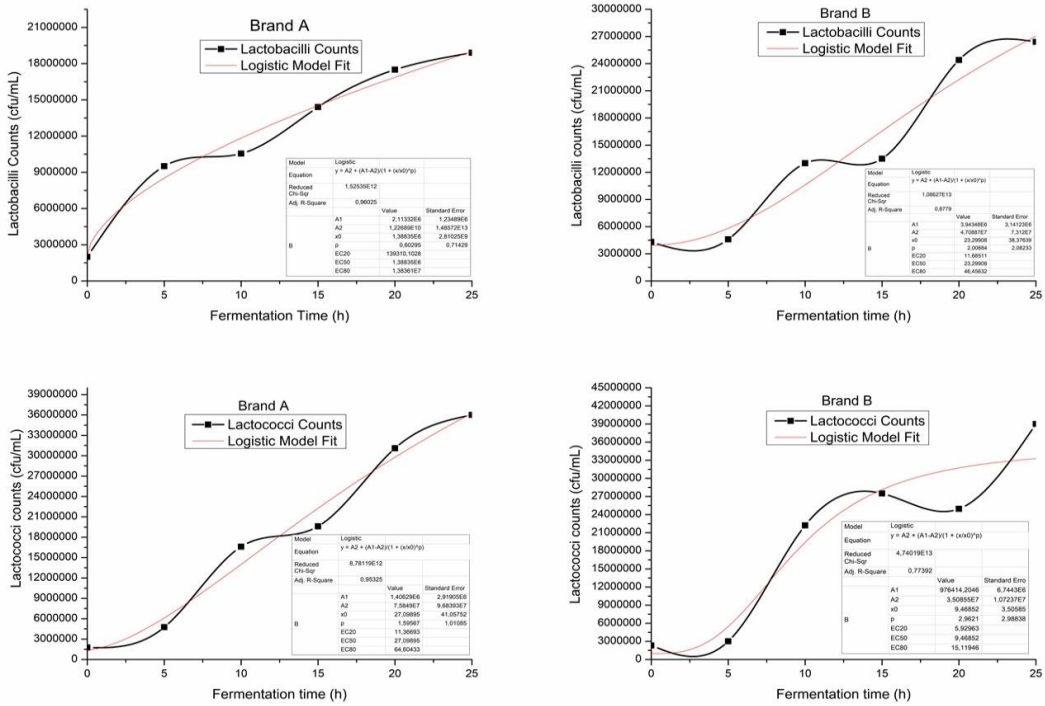

Fig.1. Microbial growth kinetics of kefir samples are produced with Brand A and B beverages. *Lactobacilli* growth kinetics and *Lactococci* growth kinetics

The changes in the number of *lactococci* bacteria during the incubation period of kefir samples are shown in Figure 1. The difference between the two brands is less pronounced for *lactococci*. Brands A and B kefir samples fit the logistic model highly; their adjusted R^2 values were 0.9533 and 0.7739, respectively. In kefir beverages produced from brand A, *lactococci* were 6.24 log CFU/mL at the beginning of fermentation and 7.56 log CFU/mL at the end of the incubation period. In brand B, *lactococci* were 6.40 log CFU/mL immediately after inoculation and 7.59 log CFU/mL at the end of incubation. The same increasing trend and numbers were observed for both productions. The *lactococci* counts in kefirs obtained from brands A and B increased

significantly until the 25th hour of fermentation (P<0.05). *Lactococci* growth in kefir samples incubated with commercial kefir starter culture increased from 5.50 log CFU/mL to 9.05 log CFU/mL, and the addition of oleaster flour and high-pressure application increased the growth rate (Gul et al., 2023). Kefir made with starter culture, the counts of *lactococci* increased from 5.80 log CFU/mL to 7.80 log CFU/mL after 24 hours of incubation (García Fontán et al., 2006). Fermented commercial Norwegian kefir samples showed a *lactococci* count of 8 log CFU/mL (Grønnevik et al., 2011).

The changes in total mesophilic aerobic bacteria count during the incubation period of kefir samples are shown in Figure 2. The kefirs obtained from brands A and B fit the logistic model, and their adjusted R^2 values were 0.9609 and 0.9887, respectively. This indicates an initial lag phase, followed by exponential growth, and then, a stationary phase in which growth halts.

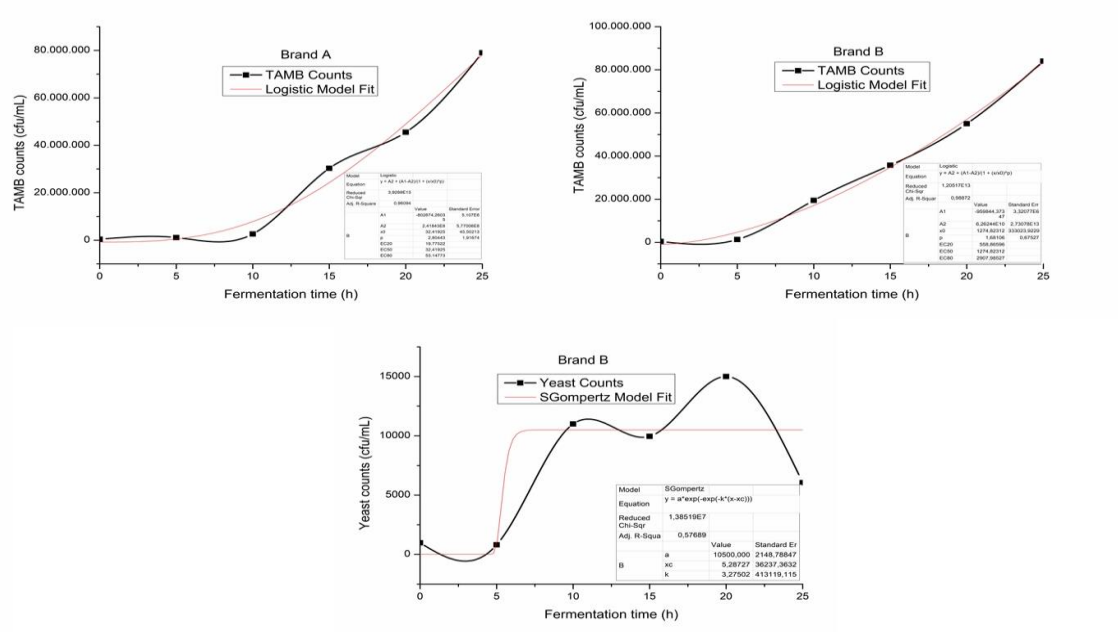

Fig.2. Microbial growth kinetics of kefir samples are produced with Brand A and B beverages. Total mesophilic aerobic bacteria growth kinetics and Yeast growth kinetics

In brand A, the total number of mesophilic aerobic bacteria was 5.52 log CFU/mL at the beginning of fermentation and 7.89 log CFU/mL at the end of the incubation period. In brand B, the total number of mesophilic aerobic bacteria was 5.63 log CFU/mL immediately after inoculation and 7.92 log CFU/mL at the end of the incubation period. This increase was significant during the fermentation until the end of incubation (P<0.05).

The variations in yeast counts of kefir samples during the incubation period are shown in Figure 2. While no yeast growth was observed in kefirs obtained from brand A, yeast change in kefirs produced from brand B was found to fit the Gompertz model than the logistic model, with a regression coefficient of 0.5769. The yeast population experiences a rapid increase, reaches a peak, and then declines slightly. In brand B, the yeast count was 2.99 log CFU/mL immediately after inoculation and 3.78 log CFU/mL at the end of incubation. A decrease in yeast count was observed after the 20th hour. This decline can be attributed to the competition for nutrients between the microorganisms. The yeast count was 3 log CFU/mL in freshly produced kefir samples (Grønnevik et al., 2011).

Yeast in kefir samples made with kefir starter culture increased from 2 log CFU/mL to 3 log CFU/mL, and hazelnut milk addition promoted the growth rate (Atalar, 2019). In another study, kefir produced with commercial starter culture was not detected after 24h incubation (García Fontán et al., 2006). Some brands refrain from using yeast-included starter cultures due to packaging problems with forming CO_2. The results reveal that using two different kefir brands showed similar microbial growth kinetics during fermentation except yeast viability.

pH change kinetics

The pH changes of kefir samples during the incubation period are shown in Figure 3. The graph obtained from hourly measurements was fitted to the logistic model, and the adjusted R^2 values were 0.9970 and 0.9916, respectively. The pH values of kefirs produced from both brands were 6.44 at the beginning of inoculation and decreased to 4.28 in both kefir samples after 24 hours of incubation. The pH values of the original commercial kefir drinks used for fermentation were 4.22 and 4.30 for brands A and B, respectively.

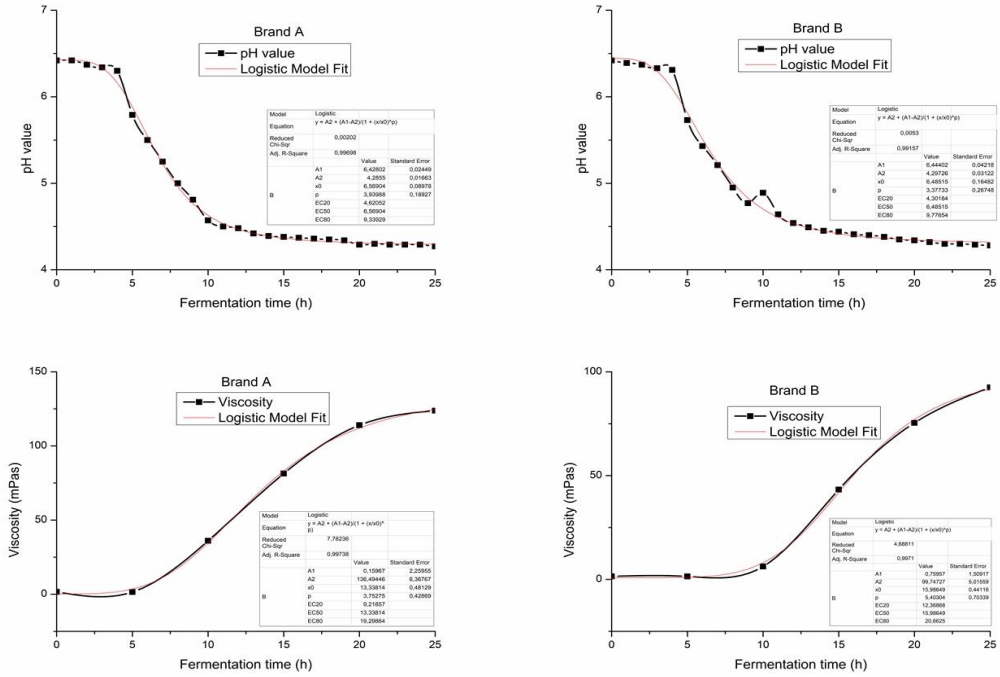

Fig.3 pH and viscosity kinetics of kefir samples produced with Brand A and B beverages

For both brands, the maximum decrease in pH values was determined to be 5 hours. The time for kefir samples to reach pH 4.5 was 11 hours for Brand A and 13 hours for Brand B. V_{max} values were determined as 9.08 and 5.83 for Brand A and B, respectively. Our findings are in agreement with those of different studies. The acidification kinetics parameters of kefir samples produced with starter culture were reported as V_{max} between 1.85 and 1.75×10^{-3} pH units per minute, T_{max} 12 hours and time to $T_{pH5.0}$ 14 hours (Atalar, 2019).

The pH values of kefir drinks produced from Saane, Hair goat and cow milk varied between 4.54 and 4.59 during fermentation (Satir and Guzel-Seydim 2015). The pH values of kefirs produced from kefir grains incubated at 25°C were 4.85-4.85 and 4.30 at 18, 24, and 48 hours, respectively (Hecer et al., 2019). V_{max}, T_{max}, and TpH5.0 values of kefir produced from the starter culture are 0.28 ± 0.01, 12, and 14, respectively (Gul et al., 2023).

Viscosity change kinetics

The viscosity changes of kefir samples during incubation are shown in Figure 3. The viscosity values of kefirs produced with brands A and B were 1.58 mPa.s and 1.44 mPa.s at the beginning of fermentation and 123.79 mPa.s and 92.49 mPa.s at the end of the incubation period. The viscosity variations of brands A and B highly fit a logistic model, with adjusted R^2 values of 0.9974 and 0.9971, respectively. There is a wide range of kefir viscosity values in the literature. Kefir produced from starter culture has a viscosity value of 42.14 mPas after fermentation (Sarica & Coşkun, 2020). Kefir made from kefir grains showed a viscosity of 225 mPas (Kök-Taş et al., 2013). The viscosity values of kefir produced with cow milk were 101.1 mPas after fermentation (Tratnik et al., 2006).

Generation study

Microbial growth, pH and viscosity changes

Lactobacilli count results of the samples in the generation study for 4 days are given in Figure 4. In kefir drinks produced from brand A, lactobacilli count was 6.45 log CFU/mL at the end of the 4th generation, which was 6.61 log CFU/mL at the first generation.

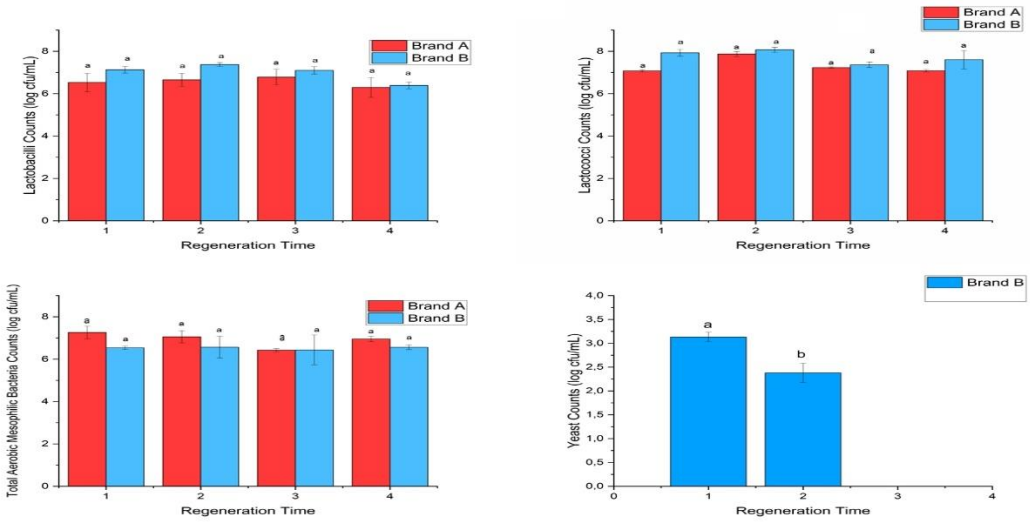

Fig.4. Microbial change of kefir samples produced with Brand A and B beverages during regeneration

In brand B, the number of lactobacilli was 6.63 log CFU/mL after the first generation and 6.46 log CFU/mL at the end of the 4th generation. The generation of kefir samples did not change the number of *lactobacilli* (P>0.05). However, a decline was observed compared to the first-time production results (The results were given in Section 3.1.1). The total number of *lactococci* in kefir beverages produced from brand A was 7.07 log CFU/mL at the first generation and 7.08 log CFU/mL at the end of the 4th generation. In brand B, *lactococci* were 7.92 log CFU/mL after the first generation and 7.6 log CFU/mL at the end of the 4th generation. As in the case of *lactobacilli*, no change in the number of *lactococci* was observed with the generation process (P>0.05). The total number of mesophilic aerobic bacteria in kefir beverages produced from brand A was 7.02 log CFU/mL for the first generation and 6.73 log CFU/mL at the end of the 4th generation. In brand B, the total number of mesophilic aerobic bacteria was 6.42 log CFU/mL after the first generation and 6.41 log CFU/mL at the end of the 4th generation. The change in the number was found to be insignificant (P>0.05). As shown in Figure 3, while no yeast growth was observed in brand A, the presence of yeast was detected in brand B only in the first and second generations. In kefir produced from brand B, the yeast count was 3.13 log CFU/mL after the first generation and 2.38 log CFU/mL after the second generation (P<0.05). In Summary, the generation

process after the first production decreased the number of microorganisms. However, no change in viability was detected in the generation processes.

The pH values measured for 4 days in the generation study are shown in Figure 5. The pH values of kefir samples produced from brands A and B slightly decreased from the first generation. The pH value of kefir drinks produced from brand A, which was 4.41 for the first generation, was determined as 4.39 pH at the end of the 4th generation. In brand B, the pH value was 4.51 after the first generation and 4.35 at the end of the 4th generation.

The viscosity of brand A kefir decreased from the first generation to the 3rd generation. However, it is seen that the viscosity increased on the 4th day. When we look at the data for brand B, we see an increase in viscosity values from the first generation.

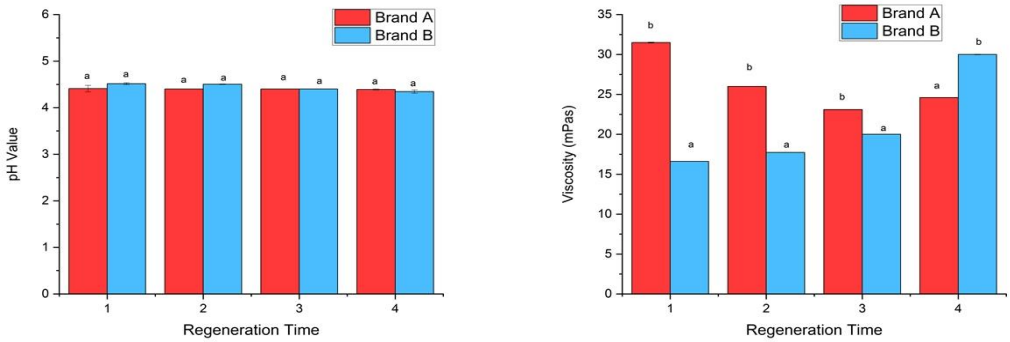

Fig.5. pH and viscosity change of kefir samples produced with Brand A and B beverages during regeneration

Aydemir (2020) purchased four commercial yogurts from the market and investigated their usability in home yogurt production. The yogurts were inoculated into fresh milk and passed four times. Similar to our findings, the time for pH values to reach 4.60 was shortened from the first generation and a lower pH value was reached on the last generation. The viscosity values produced in the first generation were the lowest compared to the other three productions. This may be related to the adaptation of microorganisms to the environment. It was revealed that commercial yogurt can be used as a starter culture in home-type yogurt production.

Storage study

Microbial growth

Microbial counts of the kefir samples in the storage study for 4 weeks are given in Table 1. The number of lactobacilli decreased during storage in kefirs produced with both brands. The number of lactobacilli was 7.73 log CFU/mL on the first day of storage and 7.02 log CFU/mL at the end of storage. In kefir produced with brand B, the number of lactobacilli was 7.86 log CFU/mL on the first day and decreased to 7.11 log CFU/mL at the end of storage. The decrease during storage was statistically significant ($P<0.05$). The decrease is lower than in Norwegian commercial kefir samples, whose lactobacilli count was 8 log CFU/mL, and 2 log decreases were detected in the numbers of lactobacilli in the 4th week of storage (Grønnevik et al., 2011). Goncu et al. (2017) determined that the lactobacilli count of kefir samples was 10.23 log CFU/mL on the 1st day of storage and 9.61 log CFU/mL on the 20th day of storage. Abdolmaleki et al., (2015) found that the lactobacilli count in kefir samples produced using milk, soy milk and whey was 8.28 log CFU/mL on the 1st day of storage and decreased to nearly 5 log CFU/mL on the 28th day of storage.

During storage, the number of lactococci in kefir produced with brand A was 7.29 log CFU/mL on the first day of storage and 6.59 log CFU/mL on

the last day of storage. In kefir produced with brand B, the number of lactococci was 7.77 log CFU/mL on the first day of storage, decreasing to 6.81 CFU/mL at the end of storage. The decreases for both brands were significant (P<0.05). This could be attributed to cell proteolysis due to the reduction in pH. Similar findings were observed in the literature. *Lactococci* counts decreased from 9.23 to 8.04 log CFU/mL during 21 days of storage in kefir samples produced with kefir grains (Kök-Taş et al., 2013). Lactococci levels decreased during storage, and the most significant decrease of approximately 1.5 log units occurred from days 7 and 14, which is statistically significant (Irigoyen, 2005). Abdolmaleki et al., (2015) reported that lactococci counts decreased by roughly 3 log during storage, which was statistically significant (P<0.01).

Table 1. Microbial results of kefir samples during storage

	Lactobacilli Counts (log CFU/mL)		Lactococci Counts (log CFU/mL)		Yeast Counts (log CFU/mL)		TMAB Counts (log CFU/mL)	
Storage Time	A Brand	B Brand	A Brand	B Brand	A Brand	B Brand	A Brand	B Brand
1. Day	7.73 ± 0.68^{aA}	7.86 ± 0.49^{aA}	7.29 ± 0.34^{bA}	7.77 ± 0.66^{aB}	N.D	4.48 ± 0.03^{C}	7.9 ± 0.14^{aA}	8.36 ± 0.07^{aA}
7. Day	7.7 ± 0.22^{aA}	7.46 ± 0.21^{bB}	7.12 ± 0.04^{bAB}	8.03 ± 0.11^{aB}	N.D	4.86 ± 0.16^{A}	7.81 ± 0.04^{bA}	8.48 ± 0.02^{aA}
14. Day	7.54 ± 0.08^{aBC}	7.13 ± 0.16^{bC}	7.24 ± 0.08^{bA}	8.21 ± 0.24^{aA}	N.D	4.72 ± 0.17^{B}	7.64 ± 0.51^{bAB}	8.28 ± 0.21^{aAB}
21. Day	7.63 ± 0.04^{aB}	7.01 ± 0.16^{bCD}	6.59 ± 0.02^{bC}	8.38 ± 0.02^{aA}	N.D	4.76 ± 0.21^{AB}	7.21 ± 0.01^{bB}	8.05 ± 0.33^{aB}
28. Day	7.02 ± 0.02^{aD}	7.11 ± 0.01^{aC}	6.59 ± 0.1^{bC}	6.81 ± 0.28^{aC}	N.D	4.85 ± 0.21^{A}	6.81 ± 0.04^{aC}	$6.81\pm0,27^{aC}$

*N.D: Not Determined. a−b Different superscript lowercase letters in rows indicate significant differences between brands in 95% of confidence (P<0.05); A-D Different superscript uppercase letters in columns indicate significant differences between storage periods in 95% of confidence (P<0.05)

While the presence of yeast could not be detected in the productions made with brand A, the yeast count in the productions made with brand B was 4.48 log CFU/mL on the first day of storage and 4.85 log CFU/mL on the last day. There was a slight increase in kefir produced from brand B during storage, which was statistically significant (P<0.05). Similar findings were observed for Norwegian kefir samples; yeasts constantly increased during storage at the end of 3 weeks. (Grønnevik et al., 2011). In productions made with brand A, the total number of mesophilic aerobic bacteria at the beginning of storage was 7.90 log CFU/mL, while on the last day, it was 6.81 log CFU/mL. In the productions made with brand B, the total number of mesophilic aerobic bacteria, 8.36 CFU/mL on the first day of storage, was found to be 6.81 CFU/mL on the last day of storage. The decrease in total mesophilic aerobic bacteria count was found to be significant on the 28th day of storage in kefir samples produced from both kefir brands (P<0.05).

Color properties

The physicochemical changes of kefir samples during the storage period are given in Table 2. While the L* value of kefir made with brand A was 84.17 at the beginning of storage, it was 83.83 on the last day of storage. A similar observation was observed in kefir made with brand B; while the L* value was 84.11 at the beginning of storage, it was found to be 83.96 on the last day of storage. These differences were found to be significant (P<0.05). No statistical difference was observed in the a* values of kefir produced with both brands throughout storage (P>0.05). The negative a* values of kefir samples were due to their slightly green color. While the b* value of kefir made with brand A was 6.07 at the beginning of storage, it was found to be 5.81 on the last day of storage. This difference was found to be significant (P<0.05). A similar observation was observed in kefir made with brand B; while the b* value was 4.02 at the beginning of storage, it was found to be 3.9 on the last day of storage (P<0.05). A significant difference was detected regarding the b* value in kefir produced from both brands. The color saturation can explain why the difference became stronger during the storage. (Czyżak-Runowska et al., 2022). Kefir samples manufactured from cow and buffalo milks by using kefir grains and starter cultures, L*

values varied between 91.80 and 92.98, a* values varied between -0.87 and -1.71, and b* values ranged between 6.47 and 10.61 (Gul et al., 2018).

Table 2. Physicochemical results of kefir samples during storage

	L*		a*		b*	
Storage Time	A Brand	B Brand	A Brand	B Brand	A Brand	B Brand
1. Day	84.17±0.01^{Ca}	84.11±0.01^{Aa}	-2.37±0.01^{Aa}	-1.62±0.02^{Aa}	6.07±0.02^{Da}	4.02±0.02^{Ab}
7. Day	84.14±0.01^{Da}	83.98±0.01^{Ba}	-0.89±3.03^{Aa}	-1.64±0.02^{Ab}	6.25±0.04^{Ba}	3.95±0.04^{Bb}
14. Day	84.89±0.01^{Aa}	83.73±0.01^{Da}	-2.37±0.02^{Ab}	-1.53±0.01^{Aa}	6.54±0^{Aa}	3.68±0.02^{Eb}
21. Day	84.19±0.01^{Ba}	83.95±0.01^{Ca}	-2.33±0.01^{Ab}	-1.39±0.02^{Aa}	6.14±0.01^{Ca}	3.81±0.01^{Db}
28. Day	83.88±0^{Ea}	83.96±0.01^{Ca}	-2.37±0.02^{Ab}	-1.57±0.35^{Aa}	5.81±0.01^{Ea}	3.9±0.02^{Cb}
	pH		Titratable Acidity (% Lactic acid)		Syneresis (%)	
Storage Time	A Brand	B Brand	A Brand	B Brand	A Brand	B Brand
1. Day	4.58±0.04^{Aa}	4.4±0.01^{Aa}	0.77±0.0^{Aa}	0.78±0.01^{Aa}	35.16±1.19^{Aa}	28.32±0.96^{Bc}
7. Day	4.46±0.01^{ABa}	4.41±0.01^{Aa}	0.75±0.02^{Aa}	0.76±0.03^{Aa}	31.34±1.9^{Ab}	28.56±0.79^{Bc}
14. Day	4.38±0.03^{Ba}	4.42±0.02^{Aa}	0.74±0.0^{Aa}	0.74±0.01^{Aa}	29.68±0.45^{Bb}	33.14±1.61^{Ab}
21. Day	4.41±0.08^{ABa}	4.42±0.05^{Aa}	0.75±0.01^{Aa}	0.75±0.01^{Aa}	29.04±0.06^{Bb}	41.42±2.01^{Aa}
28. Day	4.39±0.12^{Ba}	4.4±0.07^{Aa}	0.76±0.01^{Aa}	0.77±0.01^{Aa}	36.2±1.7^{Aa}	38.32±1.87^{Aa}

*N.D: Not Determined. a−b Different superscript lowercase letters in rows indicates significant differences between brands in 95% of confidence (P<0.05); A-D Different superscript uppercase letters in columns indicates significant differences between storage periods in 95% of confidence (P<0.05)

Syneresis

In the kefir samples produced from the kefir of brand A, syneresis values were 35.16% on the first day of storage and 36.20% on the last day of storage (Table 2). This difference was not statistically significant (P>0.05). In kefir samples produced from brand B kefir, serum separation was found to be 28.32% on the first day of storage and 38.32% on the last day of storage. The increase that occurred, especially after the 14th day, was found to be statistically significant. The syneresis values between the two brands may differ in the exopolysaccharide production levels of used starter cultures. The change in serum separation values of kefirs Kefir produced from cow's milk between the 1st and 14th days was found to be significant (P<0.05), while made from goat's milk was found to be insignificant throughout storage (P>0.05) (Sarica & Coşkun, 2020). Kefir samples made from kefir grains varied between 25.71-30.12%, and the kefir samples made with starter culture varied between 23.50-28.50% (Yousefvand et al., 2022).

pH and titratable acidity

The acidity change of kefir samples throughout storage is given in Table 2. The pH value of the production with brand A was 4.58 at the beginning, and the pH was found to be 4.39 on the last day of storage due to the metabolic activity of microorganisms, especially lactic acid bacteria, that metabolize lactose and nitrogenous substances in kefir. The pH value of the production made under brand B was 4.4 on the first and last day of storage. While the pH value of the production made with brand A decreased during storage (P<0.05), no change was observed in the values of brand B (P>0.05). From the first to the last day of storage, the pH of the fermented milk drink from Brazilian milk kefir gradually decreased from 6.55 to 4.31 (P<0.05) (Leite et al. 2013). The pH values of kefirs were 4.50, 4.10, and 4.10 on the 1st, 7th, and 15th days of storage, respectively (Öner et al., 2010).

The titratable acidity of kefir samples produced from brand A was 0.77% at the beginning of storage and 0.76% on the last day of storage. The

change during storage was insignificant (P>0.05). In the kefir samples produced from brand B, titratable acidity was found to be 0.78% on the first day of storage and 0.77% on the last day, and the change during storage was insignificant (P>0.05). The % lactic acid values of kefir samples were determined as 0.89% on the 1st day of storage, 0.84% on the 7th day and 0.92% on the 21st day of storage (Kök-Taş et al., 2013). The titratable acidity value of kefir samples produced from cow and buffalo milk was between 0.64% and 0.76% on the 1st day. The titratable acidity value did not differ between kefir samples during storage (Gul et al., 2015). The % lactic acid values of kefirs were measured as 0.68, 0.78 and 0.87 during the 1st, 10th, and 20th days of storage, respectively (Goncu *et al.* 2017).

Sensory properties

The sensory scores for kefir samples produced with commercial kefir beverages are presented in Fig.6. An increase in taste and aroma values was observed in kefir samples produced from brand A compared to the first day of storage, and the highest score was detected in the samples on the 7th day. The taste and aroma scores in the kefir samples produced from brand B showed a decreased tendency during storage. The highest score was on the 1st day of storage, while the taste and aroma scores decreased as storage progressed (P<0.05). While the consistency scores of kefir samples produced from brand A enhanced during storage (P<0.05), brand B samples remained constant throughout storage (P>0.05). While there was an increase in the color and appearance scores of kefir samples produced from brand A throughout storage (P<0.05), a significant decrease was observed in kefir samples produced from brand B on the 28th day of storage (P<0.05). In the samples obtained from both kefir brands, the lowest overall liking score was determined on the first day of storage.

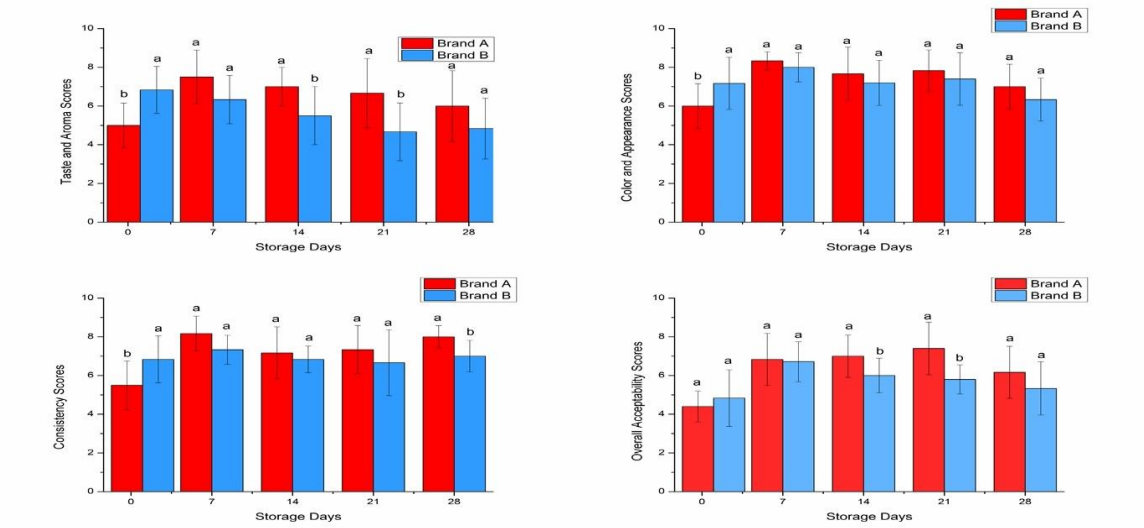

Fig.6. Sensory evaluation of kefir samples produced with Brand A and B beverages during storage

While the highest overall acceptability score for kefir samples produced from brand A was obtained on the 21st day of storage, the highest score was determined for kefir samples produced from brand B on the 7th day of storage. Akbörü (2019) produced kefir using different commercial cultures. The general acceptability of kefir samples received the highest score in the 7th storage. The differences between kefirs were statistically significant throughout storage. Goncu et al. (2017) determined that while the general acceptability of kefir samples increased during the first 10 days of storage, but decreased thereafter. The difference may be related to the growth of acidity and the reduction in the content of aroma compounds (such as acetaldehyde) in kefir samples during storage.

CONCLUSION

Within the scope of this study, the potential for producing homemade kefir from commercial kefir beverages was investigated. Fermentation, generation and storage steps were studied to characterize the kefir samples. The fermentation growth kinetics were fitted to the logistic model in *lactobacilli, lactococci*, TAMB, pH and viscosity values with 0.8779-0.9973 adjusted R^2. For both brands, the maximum decrease in pH values was determined to be 5 hours. The time for kefir samples to reach pH 4.5 was 11 hours for Brand A and 13 hours for Brand B. The generation of kefir samples did not change the number of *lactobacilli*, *lactococci*, and TAMB counts during the 4th passing. In kefir produced from brand B, the yeast count was 3.13 log CFU/mL after the first generation and decreased to 2.38 log CFU/mL in the second generation. Yeast was not detected in the third and fourth generations. During storage, *lactobacilli*, *lactococci*, and TAMB counts decreased significantly (P>0.05). While no yeast growth was observed in the kefir samples produced from brand A, there was a slight increase in the kefir samples produced from brand B, but this increase was not found to be statistically significant (P>0.05). While no statistical difference was observed in the a* values of kefir produced with both kefir brands throughout storage (P>0.05), a significant difference was detected in the b* and L* values. During storage, syneresis increased in both kefir brands, indicating structural weakness. The kefir drink produced with Brand B meets the Codex Alimentarius recommendations for fermented milk (Codex Alimentarius Commission, 2003), i.e., the total microorganism count should be at least 10^7 CFU/mL and the yeast count at least 10^4 CFU/mL. As the yeast was not observed in Brand A, kefir drinks produced with Brand A did not provide the required yeast values prescribed by the Codex Alimentarius. Despite structural problems, this technique can be adequate for producing kefir beverages in homemade conditions. However, these commercial kefirs can be collected from market shelves at different periods, and fluctuations in product temperature make it difficult to create a standardized product in terms of quality characteristics in the final product.

CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ozlem Şahin: Formal Analysis. Ilyas Atalar: Writing – original draft, Conceptualization, Methodology. Seyma Betul Encu: Formal Analysis. Ibrahim Cakir: Writing – review & editing, Supervision.

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