



Comparison of different animal milks on microbiological, physicochemical, sensory properties, and volatile component profile of Kefir

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Abstract

In this study, the effects of cow, buffalo, ewe, and goat milk on the microbiological, physicochemical, sensory properties, and volatile component profile of kefir were examined during storage. Key microbial counts, including lactic cocci, lactic bacilli, and yeast, varied among kefirs, with lactic bacilli and lactic cocci decreasing over time, while yeast counts increased. The titratable acidity ranged from 1.12 to 1.16%, and pH values varied between 4.51 and 4.83. Sensory analysis showed that cow milk kefir received the highest consumer appreciation, while goat milk kefir was least favored. Notably, ewe milk kefir contained the highest benzoic acid content. Principal component analysis identified distinct clusters in the volatile profiles of the kefirs. Our results suggest that buffalo and ewe milk kefirs may be viable alternatives to cow milk kefir, with potential for consumer acceptance based on sensory properties.

Keywords Kefir · Milk type · Physicochemical property · Volatile compound

Abbreviations

AOAC	Association of Official Agricultural Chemists	GC-MS	Gas Chromatography-Mass Spectrometry
AHC	Agglomerative Hierarchical Clustering	DRBC	Dichloran–Rose Bengal Chloramphenicol Agar
APC	Aerobic Plate Count	EK	Ewe Milk Kefir
A _x	Area of Volatile Components	HPLC	High Pressure Liquid Chromatography
A _{st}	Area of Internal Standard	IS	Internal Standard
CFU	Colony Forming Unit	GK	Goat Milk Kefir
BK	Buffalo Milk Kefir	K	Consistency Coefficient
C	Quantitative Amount	ISO	International Organization for Standardization
CK	Cow Milk Kefir	LAB	Lactic Acid Bacteria
C _{st}	Amount of n-Butanol	mL	Milliliter
		MRS	De Man, Rogosa and Sharpe Agar
		PCA	Plate Count Agar
		PCA	Principal Component Analysis
		PDMS-SPME	Carboxene-Polydimethylsiloxane Solid-Phase Microextraction
		RPM	Revolutions Per Minute
		SPSS	Statistical Package for the Social Science
		SO	Dilution Rate
		TS	Turkish Standards
		UV	Ultra Violet
		VRBA	Violet Red Bile Agar

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VWD Variable Wavelength Detector
 WI White Index

microbiological, physicochemical, sensory, and volatile component profiles during 28 days of cold storage (4 °C).

Introduction

Kefir is a sour, self-carbonated, and slightly alcoholic fermented milk product originating from the Caucasus mountains [1, 2]. According to the Codex Alimentarius Commission (2022), kefir is defined as fermented milk products that follow starter cultures prepared from kefir grains—*Lactobacillus kefiri*, a species of the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter*—which grow in a strongly specific relationship. Kefir grains constitute both lactose fermenting yeasts (*Kluyveromyces marxianus*) and nonlactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguus*). The codex also states that milk protein in kefir should be at least 2.7%, fat should be less than 10%, the titration acidity should be at least 0.6% lactic acid, the number of microorganisms should be at least 10^7 cfu/mL, and the number of yeast should be at least 10^4 cfu/mL [3].

Traditionally, kefir is produced using kefir grains, which have a cauliflower-like appearance, range in size from 3 to 35 mm, and consist of a complex community of bacteria and yeasts embedded in an exopolysaccharide matrix known as kefiran [1, 4]. These grains host a diverse microbiota, including lactic acid bacteria (LAB), acetic acid bacteria, and yeasts, which grow in a symbiotic relationship [5]. On the other hand, with the increase in kefir production and consumption worldwide, kefir is being produced commercially from starter cultures [6].

The content of kefir varies on the basis of the microorganisms used in production, milk type, fermentation process and temperature, and storage conditions [7]. In addition to cow milk, milk from other mammal species, such as buffalo [8], ewe [9], and goat [10], and vegetable milk, such as peanuts and walnuts [11] can also be used in kefir production. Kefir fermentation can produce lactic acid, ethyl alcohol, CO₂, bacteriocins, vitamins, antioxidants, exopolysaccharides, various enzymes, and amino acids [12]. Studies have revealed that kefir has therapeutic effects on many diseases, such as cardiovascular diseases [13], cancer [14], and diabetes [15], and can also have antiviral [16] and antioxidant [9] effects.

This study compared milk from four different animals in terms of kefir content. The benzoic acid content and volatile compounds of kefir samples were investigated and compared. Since studies on this topic are limited, we aimed to prepare kefir from cow, buffalo, ewe, and goat milk using commercial starter culture and compare their

Materials and methods

Materials

Raw cow milk was purchased from the Food and Livestock Application and Research Centre (Atatürk University, Erzurum, Türkiye) while raw buffalo, ewe, and goat milk were supplied by farms in Erzurum, Türkiye. The raw milk was transferred to the laboratory at 4°C, and kefirs were produced immediately. The production of the kefir samples was conducted via commercial starter culture (Chr. Hansen, eXact® KEFIR 2, Hørsholm, Denmark), which included *Lactococcus lactis* subsp. *cremoris/lactis*, *Leuconostoc* sp., *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Streptococcus thermophilus*, and *Debaryomyces hansenii* bacteria.

Kefir production process

The productions of kefir samples from cow, buffalo, ewe, and goat milk was performed according to the methods reported by Aktaş et al. [17] and summarized in Fig. 1. Two independent batches of kefir were produced (biological replicates). Briefly, raw milk was pasteurized at 90°C (10 min), then cooled to 25°C. Whole direct set cultures were added to 100 mL (pasteurized) milk for incubation and used at a 3% (v/v) ratio as the starter culture from the mixture. The milk was divided into jars and incubated at 25°C for 20 h for kefir fermentation. The jars were covered with a sterile cheese-cloth, secured with a rubber band, and incubated in the dark. The produced cow milk kefir (CK), buffalo milk kefir (BK), ewe milk kefir (EK), and goat milk kefir (GK) samples were stored at 4°C for 28 days. The quality parameters of the samples, such as their microbiological, physicochemical and sensory properties, volatile component profile, and benzoic acid content were investigated during storage on days 1, 14, and 28.

Microbiological analyses

The aerobic plate count (APC), lactic bacilli, lactic cocci, coliform bacteria, and yeast–mold content of the milk samples were investigated to establish their microbiological properties over a storage period of 28 days. The samples were diluted with Ringer's solution (Merck, Darmstadt, Germany) according to the serial dilution method [18]. Plate Count Agar (PCA; Merck) was used to enumerate aerobic plate counts following TS EN ISO 4833-1. De Man, Rogosa

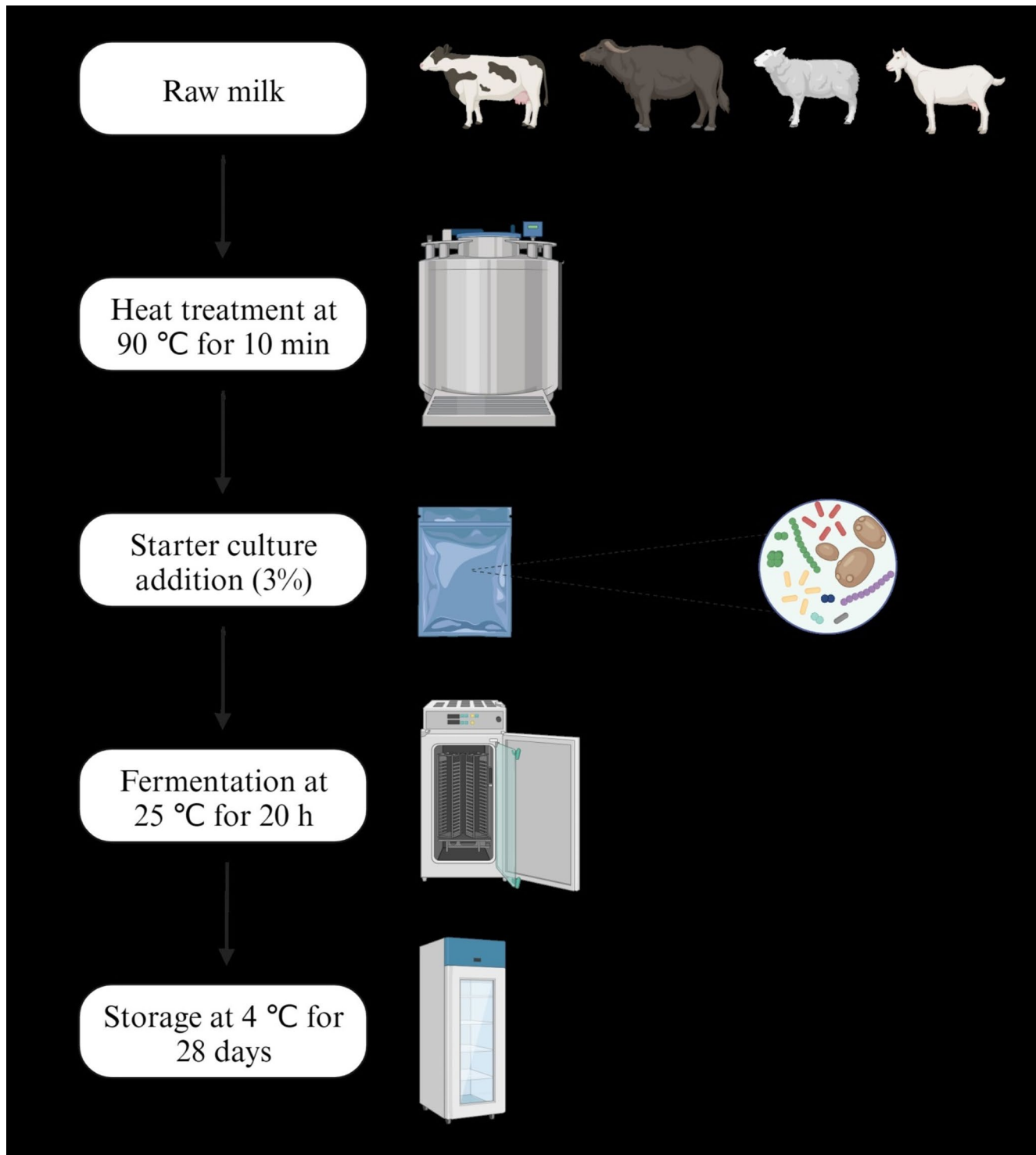


Fig. 1 Kefir production process used in this study (Created in BioRender. Aktaş, H. (2024) <https://BioRender.com/q92l891>)

and Sharpe Agar (MRS; Merck) was used for lactic bacilli, and M17 agar was used for lactic cocci enumeration [19]. Violet Red Bile Agar (VRBA; Merck) was used for coliform bacteria according to TS ISO 4832, and Dichloran–Rose Bengal Chloramphenicol Agar (DRBC; Merck) was used

for yeast and mold counts following TS ISO 21527-1:2008. The petri dishes, including PCA, M17, VRBA, and DRBC dishes, were incubated at 30°C for 72 h (aerobically), at 30°C for 24 h, at 37°C for 24 h, and at 25°C for 5 days, respectively, while MRS agar was incubated anaerobically

at 30°C for 48–72 h. A microscopic examination of five typical colonies cultivated on MRS and M17 media was performed to confirm bacterial growth.

Physicochemical properties

The titratable acidity (% lactic acid) and pH (Hanna instruments, Woonsocket, USA) of the kefir samples were recorded according to the AOAC [20] method at 1, 14, and 28 days of storage. The luminosity (L^*), red/green value (a^*), and blue/yellow (b^*) parameters of the kefir samples were measured via a colorimeter (Minolta, CR-400) and the measurements were performed in triplicate. Before measurement, the machine was calibrated using a white plate. The saturation (C^*) reported by Cecchini et al. [21], hue angle (H°) reported by McLellan et al. [22], and white index (WI) reported by Kurt and Atalar [23] were determined via the respective study methods. The WI equation is as follows:

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

The viscosity values of the samples were recorded with a viscometer (Brookfield, Model DV-II, Stoughton, MA, USA) and the measurements were performed with spindle a number of 5 at 5, 10, 20, 50, and 100 rpm. The rheological parameters of the kefir samples were calculated with the equation of the power law model. In this equation, the apparent viscosity (Pa.s), coefficient consistency (Pa.sⁿ), shear rate (rpm), and flow behavior index were indicated with η , K , γ , and n symbols, respectively [24]. The power law equation is as follows:

$$\eta = K\gamma^{(n-1)}$$

Volatile component profile

Extraction of volatile components

The homogenized samples (4 g) were weighed into a 20 mL solid phase microextraction (SPME) vial and 2 g of NaCl was added. The mixture was dissolved in a shaker and then placed into 75 μ m-carboxene-polydimethylsiloxane (PDMS)-SPME vials, which were previously conditioned at 250 °C and stored at 80°C for 45 min. At this time, the vials were placed in a gas chromatography-mass spectrometry (GC-MS) injection block and kept at 250 °C for 10 min [25].

Gas chromatography-mass spectrometry conditions

The volatile components of the samples were detected with an Agilent Technologies 5975 GC-MS instrument. Helium was the carrier gas, with a flow rate of 1.2 mL/min. The kefir samples were passed through an HP-5 ms capillary GC column (30 m $\times$ 0.25 mm ID, 0.2 μ m; Agilent Technologies). The oven temperature was adjusted to 40°C for 1 min, increased to 50°C at 10°C/min increments, maintained for 1 min, increased to 60°C at 1°C/min increments, increased to 190°C at 10°C/min, and maintained for 10 min. The injection temperature and the transfer line temperature were 250°C. The mass detector temperature was 230°C in electron ionization mode at 70 eV, and the ion source temperature was 230°C. The chromatograms were recorded by monitoring the total ion current in the mass range of 35–450 m/z [25].

Calculation of volatile components

The amount of each component was calculated by using n-butanol (PHR1209, Sigma Aldrich Co., St. Louis, MO, USA) internal standard (IS) with a relative response factor of 1. The quantitative amount was calculated with the following formula, and the results are given in μ g/mL:

$$C = (A_x \times C_{st}/A_{st}) \times SO$$

where C represents the quantitative amount (μ g/mL), A_x and A_{st} represent the areas of volatile components and the IS, respectively, C_{st} represents the amount of n-butanol in μ g/mL, and SO represents the dilution rate [25].

Benzoic acid content

Benzoic acid (PHR1050, Sigma Aldrich) amounts within the samples were detected via high-performance liquid chromatography (HPLC, Agilent Technologies) according to the validated method from Aktaş et al. [17]. The HPLC system consists of a 1260 quantitative pump, 1260 infinity standard autosampler, and 1260 VWD UV detector. For extraction, 5 g of sample was homogenized with methanol–water (35:65 ratio), and filtered into vials with filter paper. The injection volume was 20 μ L and at the mobile phase, acetate buffer–methanol (ratio 70:30, v/v) was introduced into the system with an isocratic flow of 0.6 mL/min. Chromatographic separation was tested on a Spherisorb ODS2 column (250 mm $\times$ 4.6 mm) with a particle size of 5 μ m, and benzoic acid was detected via a UV detector at 229 nm in the samples.

Sensory analysis

Seven semi trained panelists performed the sensory tests on the samples. The kefir samples were served on glasses and were evaluated according to a 9-point hedonic scale whereby 1 was extremely disliked and 9 was extremely liked in terms of color, consistency, gas formation, acidity, flavor, odor, and overall acceptability [26]. The sensory analysis was approved by the Gümüşhane University Research Ethics Committee (2022/6 and 26/10/2022) and ethical permission was granted.

Statistical analysis

All the data were evaluated via SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). The data are presented as the means $\pm$ standard deviations from two biological and three technical replicates, with a statistical significance of $p < 0.05$. The differences among variances and groups were determined via one-way ANOVA and Duncan's multiple comparison analysis.

Results and discussion

Microbiological properties

The APC, lactic bacilli, lactic cocci, coliform bacteria, yeast, and mold contents of the samples were determined at days 1, 14, and 28 of milk storage, and the obtained data are given in Table 1. The APCs of all the samples ranged from 7.23 to 8.89 log cfu/mL. The APC contents of almost

all the samples decreased during storage ($p < 0.05$). The highest APC content was detected in the CK on day 1 of storage, in the BK and EK on day 14, and in the BK on day 28 ($p < 0.05$). Setyawardani et al. [27] reported that the total bacteria count of kefir produced with different ratios of colostrum added ranged from 7.04 to 8.23 log cfu/mL. On the other hand, in another study by Aroua et al. [28], the total aerobic mesophilic bacteria counts of kefir samples (from cow and donkey milk) were 4.80–4.97 and 4.12–4.71 log cfu/mL, respectively. According to the results of these studies, the APC in kefir samples might vary. Similarly, Arslan [1] reported that the microbial population in kefir can vary because of factors, such as grain origin and the bacterial culture method used. It is possible that this variation can be high when traditional methods are used, but is lower with commercial culture methods.

The lactic bacilli and lactic cocci counts ranged from 7.01 to 8.72 and 7.42–8.77 log cfu/mL, respectively. In our study, the lactic bacilli and lactic cocci counts in almost all the samples decreased during storage ($p < 0.05$). Conversely, the yeast counts of the kefir samples were between 3.01 and 4.77 log cfu/mL and increased during storage ($p < 0.05$). Similarly, Grønnevik et al. [29] reported that LAB counts in kefir samples decreased, whereas the yeast count increased throughout the storage period. Since yeasts are more resistant to higher salt and more acidic conditions than bacteria are, this change in microbial composition might be due to the increased acidity of kefir samples during storage [30]. On the other hand, Witthuhn et al. [31] reported that the LAB count could range from 4.81 to 8.93 log cfu/mL, whereas that of yeast could range from 5.18 to 8.57 log cfu/mL. In addition, Kim et al. [32] reported that the

Table 1 Some microbiological properties of the Kefir samples (log cfu/mL)

Microbiological parameters	Storage (days)	CK	BK	EK	GK
APC	1	8.89 $\pm$ 0.06 ^{Aa}	8.51 $\pm$ 0.06 ^{Ba}	8.44 $\pm$ 0.25 ^{Ba}	8.70 $\pm$ 0.06 ^{ABa}
	14	7.96 $\pm$ 0.01 ^{Bb}	8.61 $\pm$ 0.01 ^{Aa}	8.32 $\pm$ 0.20 ^{Aa}	7.70 $\pm$ 0.08 ^{Bb}
	28	7.35 $\pm$ 0.12 ^{ABc}	7.72 $\pm$ 0.12 ^{Ab}	7.42 $\pm$ 0.14 ^{ABb}	7.23 $\pm$ 0.26 ^{Bb}
Lactic bacilli	1	8.49 $\pm$ 0.24 ^{Aa}	8.69 $\pm$ 0.06 ^{Aa}	8.57 $\pm$ 0.16 ^{Aa}	8.72 $\pm$ 0.10 ^{Aa}
	14	7.85 $\pm$ 0.13 ^{Ab}	7.70 $\pm$ 0.04 ^{Ab}	7.66 $\pm$ 0.16 ^{Ab}	7.71 $\pm$ 0.15 ^{Ab}
	28	7.01 $\pm$ 0.04 ^{Bc}	7.26 $\pm$ 0.08 ^{ABc}	7.44 $\pm$ 0.14 ^{Ab}	7.02 $\pm$ 0.09 ^{Bc}
Lactic cocci	1	8.77 $\pm$ 0.04 ^{Aa}	8.74 $\pm$ 0.21 ^{Aa}	8.54 $\pm$ 0.36 ^{Aab}	8.46 $\pm$ 0.23 ^{Aa}
	14	8.62 $\pm$ 0.04 ^{Aa}	8.69 $\pm$ 0.08 ^{Aa}	8.63 $\pm$ 0.13 ^{Aa}	8.49 $\pm$ 0.04 ^{Aa}
	28	7.45 $\pm$ 0.09 ^{Bb}	8.03 $\pm$ 0.11 ^{Ab}	7.80 $\pm$ 0.15 ^{Ab}	7.42 $\pm$ 0.11 ^{Bb}
Coliform bacteria	1	< 1	< 1	< 1	< 1
	14	< 1	< 1	< 1	< 1
	28	< 1	< 1	< 1	< 1
Yeast	1	3.88 $\pm$ 0.10 ^{Aa}	3.47 $\pm$ 0.01 ^{ABc}	3.44 $\pm$ 0.20 ^{Bc}	3.44 $\pm$ 0.20 ^{Bb}
	14	3.96 $\pm$ 0.04 ^{Aa}	3.80 $\pm$ 0.08 ^{Ab}	3.92 $\pm$ 0.12 ^{Ab}	3.01 $\pm$ 0.04 ^{Bb}
	28	3.84 $\pm$ 0.01 ^{Ca}	4.76 $\pm$ 0.13 ^{Aa}	4.77 $\pm$ 0.04 ^{Aa}	4.42 $\pm$ 0.14 ^{Ba}
Mould	1	< 2	< 2	< 2	< 2
	14	< 2	< 2	< 2	< 2
	28	< 2	< 2	< 2	< 2

The data were presented as the mean $\pm$ standard deviation from three replicate analyses. A-C and a-c present the statistical significance ($p < 0.05$) between the results in the same line and column, respectively. CK: Cow milk kefir, BK: Buffalo milk kefir, EK: Ewe milk kefir, GK: Goat milk kefir

Table 2 Some physicochemical properties of cow, Buffalo, Ewe, and goat milk used in the study

Physicochemical parameters	Raw milks			
	Cow	Buffalo	Ewe	Goat
Fat (%)	3.10	5.90	6.15	3.50
Non-fat dry matter (%)	8.20	9.35	9.90	8.55
Specific gravity (g/mL)	1.0267	1.0313	1.0347	1.0271
Protein (%)	3.05	3.85	4.55	3.15
pH	6.71	6.74	6.80	6.59
Freezing point (°C)	-0.569	-0.661	-0.788	-0.603

LAB counts of kefir produced via traditional and backslowing methods were 9.73 and 9.02 log cfu/mL, respectively. In another study by Kazou et al. [33], mesophilic LAB and yeast counts of traditional and industrial kefir samples produced from cow, buffalo, and goat milk varied from 6.38 to 9.60 and <2–7.80 log cfu/mL, respectively. The data obtained from our study corroborate the findings from these published studies. According to the Codex Alimentarius Commission [3], the number of starter cultures added to kefir should not be less than 7 log cfu/mL and the number of yeasts should not be less than 4 log cfu/mL. In this study, both the lactic bacilli and lactic cocci counts of kefir were greater than 7 log cfu/mL during storage, in compliance with the Codex. However, yeast counts remained below 4 log cfu/mL in most samples, indicating a potential limitation in meeting the minimum yeast requirement.

Coliform bacteria and mold were not detected in the kefir samples. Similar results were reported by Aktaş et al. [17].

These results showed that the pasteurization process eliminated coliform bacteria, as also stated by Czarina et al. [34] and Aktaş and Çetin [35]. However, some studies suggest that coliform bacteria could be present in homemade kefir [36] and kefir produced with raw milk [34]. These data highlight the importance of using aseptic conditions and the pasteurization process for kefir production. These findings suggest that while kefir was generally microbiologically safe and met LAB requirements, improvements in fermentation conditions may be needed to achieve optimal yeast levels and full Codex compliance.

Physicochemical properties

Some physicochemical features of the raw milk are shown in Table 2, and the titratable acidity and pH values of the kefir are given in Fig. 2. The titratable acidity of the samples varied between $1.12 \pm 0.04\%$ and $1.66 \pm 0.04\%$ for lactic acid. With respect to titratable acidity, all kefir samples were in accordance with the codex (minimum 0.6% lactic acid). The acidity of the CK, EK, and GK samples did not change ($p > 0.05$) during storage, whereas the acidity of the BK sample increased ($p < 0.05$). The highest acidity rates were detected in the EK sample ($p < 0.05$) during storage. In addition, the pH of the samples varied between 4.51 ± 0.01 and 4.83 ± 0.00 . Although the pH of BK decreased throughout the storage period ($p < 0.05$), the highest pH values were also detected in the BK sample ($p < 0.05$). Aktaş et al. [17]

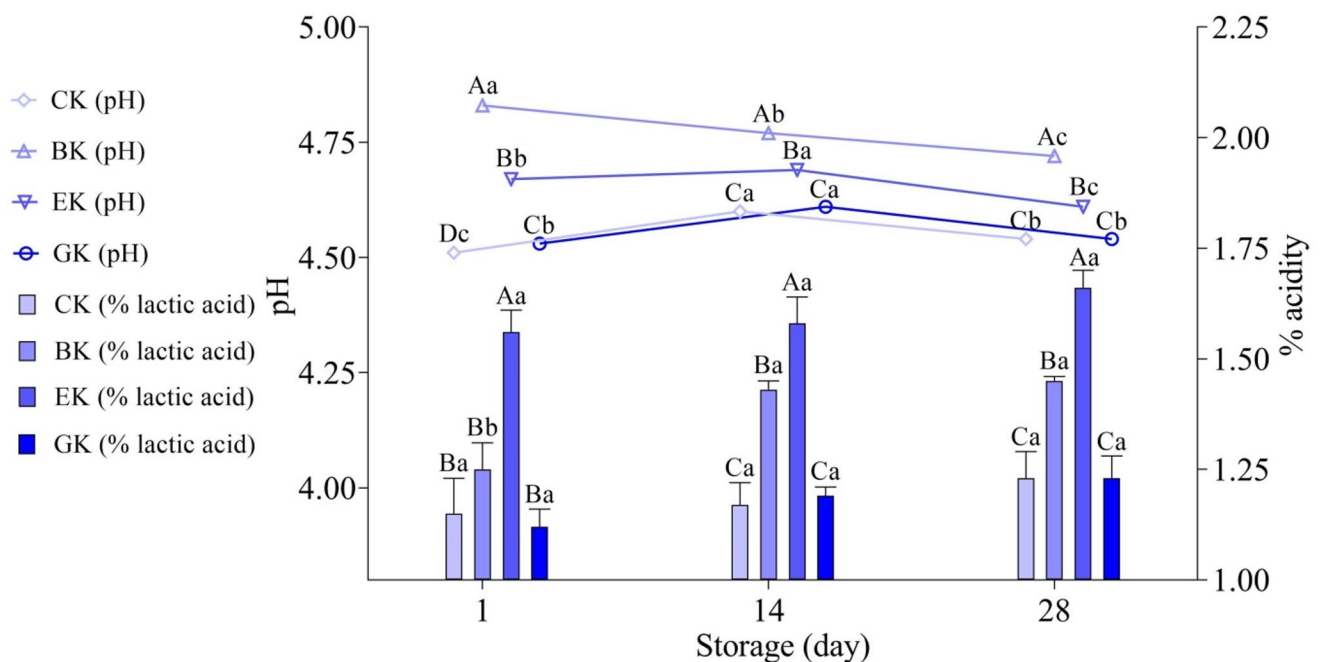


Fig. 2 Titratable acidity (% lactic acid) and pH of the kefir samples during the storage. (The data were presented as the mean $\pm$ standard deviation from three replicate analyses. A–C indicates statistical differences in different samples at the same day, a–d indicates statistical

differences in same sample at different storage days. CK: Kefir produced using cow milk, BK: Kefir produced using buffalo milk, EK: Kefir produced using ewe milk, GK: Kefir produced using goat milk)

similarly reported that the pH values of spreadable kefir samples produced from different types of animal milk varied from pH 4.51–4.83 and the acidity values ranged from 0.98 to 1.61% lactic acid. Conversely, Gul et al. [8] reported that the pH values of cow and buffalo milk kefir produced from kefir grain and starter cultures ranged from pH 4.26–4.64 and the acidity ranged from 0.64 to 0.76% lactic acid. The differences between these studies could be due to differences in culture, milk type, or fermentation time [1].

Colorimetric parameters

The values of the colorimetric parameters are given in Table 3. The L^* and WI of the BK samples were greater than those of the CK and GK samples during storage ($p < 0.05$). Furthermore, the L^* and WI values of the CK and GK samples decreased over the 28 days of storage, whereas the values of BK and EK increased ($p < 0.05$). The differences in the a^* , b^* , and C^* values were not statistically significant ($p > 0.05$) between the BK and EK samples during storage. The a^* , b^* , and C^* values of CK were lower than those of BK at days 14 and 28 of storage ($p < 0.05$). The H° values of EK and GK decreased on day 14 of storage but then increased at the end of storage ($p < 0.05$). On the other hand, the H° value of BK did not change during storage ($p > 0.05$). Similar results have been reported [37, 38]. The differences in the colorimetric parameters might have been caused by kefir produced with different milks and differences in their riboflavin, fat [37], and carotene contents [17].

Viscosity and rheological properties

The viscosity values of BK were the highest at 50 and 100 rpm during storage day 1. In contrast, the viscosity values of EK were the highest during storage, except on day 1 (Table 4). The viscosity values at 50 and 100 rpm of all the samples were lower on day 28 of storage than they were during the first days of storage. Tratnik et al. [39] and Sarica and Coşkun [38] reported that the viscosity and K values of cow kefir were greater than those of goat kefir. Tratnik et al. [39] determined a decrease in viscosity during storage in all samples, whereas Tratnik et al. [39] and Sarica and Coşkun [38] reported an increase. Gul et al. (2018) reported that the viscosity and consistency coefficient (K) values of cow kefir were lower than those of buffalo kefir, which is similar to our results.

The K values decreased in all the samples on day 28 of storage (Table 4). The highest K values were observed in BK on days 1 (51.89 Pa.sⁿ) and 14 (49.80 Pa.sⁿ) of storage, whereas in EK, it was on day 28 (22.36 Pa.sⁿ). In contrast, for all kefir samples with n values between 0 and 1, their flow behavior was pseudoplastic. GK had the lowest viscosity at 50 and 100 rpm and K values, while the n values were the highest throughout the storage period ($p < 0.05$).

Tratnik et al. [39] and Sarica and Coşkun [38] reported that the viscosity and K values of cow kefir were greater than those of goat kefir. Tratnik et al. [39] reported a decrease in viscosity during storage, whereas Sarica and Coşkun [38] reported an increase in viscosity. Gul et al. (2018) [37] reported that the viscosity and K values of cow kefir were lower than those of buffalo kefir. These results were similar to our data. The flow behavior of the kefir samples was

Table 3 Colorimetric properties of the Kefir samples

Parameters	Storage (days)	CK	BK	EK	GK
L^*	1	89.07 ± 0.25 ^{Ba}	90.21 ± 0.10 ^{Aa}	90.17 ± 0.04 ^{Aa}	89.00 ± 0.06 ^{Ba}
	14	88.65 ± 0.03 ^{Cab}	89.52 ± 0.09 ^{Ab}	88.93 ± 0.04 ^{Bb}	87.64 ± 0.11 ^{Db}
	28	88.26 ± 0.20 ^{Bb}	90.20 ± 0.04 ^{Aa}	90.28 ± 0.05 ^{Aa}	83.57 ± 0.04 ^{Cc}
a^*	1	-3.76 ± 0.28 ^{Aa}	-3.21 ± 0.25 ^{Aa}	-3.14 ± 0.48 ^{Aa}	-3.35 ± 0.12 ^{Aa}
	14	-4.21 ± 0.03 ^{Ca}	-3.58 ± 0.02 ^{Aa}	-4.17 ± 0.04 ^{Ca}	-3.90 ± 0.01 ^{Bb}
	28	-4.26 ± 0.01 ^{Ba}	-3.38 ± 0.06 ^{ABa}	-3.02 ± 0.62 ^{Aa}	-4.22 ± 0.11 ^{Bc}
b^*	1	6.71 ± 0.91 ^{Ab}	6.09 ± 0.60 ^{Aa}	6.30 ± 1.17 ^{Aa}	5.76 ± 0.34 ^{Ab}
	14	8.54 ± 0.01 ^{Ba}	6.84 ± 0.03 ^{Da}	9.61 ± 0.22 ^{Aa}	7.47 ± 0.07 ^{Ca}
	28	8.88 ± 0.01 ^{Aa}	6.61 ± 0.11 ^{Ba}	6.41 ± 1.41 ^{Ba}	7.33 ± 0.46 ^{ABa}
C^*	1	7.68 ± 0.92 ^{Ab}	6.87 ± 0.64 ^{Aa}	7.04 ± 1.27 ^{Aa}	6.58 ± 0.24 ^{Ab}
	14	9.51 ± 0.00 ^{Ba}	7.71 ± 0.03 ^{Da}	10.47 ± 0.22 ^{Aa}	8.42 ± 0.07 ^{Ca}
	28	9.84 ± 0.01 ^{Aa}	7.42 ± 0.13 ^{Ba}	7.08 ± 1.53 ^{Ba}	8.45 ± 0.45 ^{ABa}
H°	1	119.25 ± 1.48 ^{Aa}	117.73 ± 0.53 ^{ABa}	116.55 ± 0.78 ^{Ba}	120.13 ± 0.53 ^{Aa}
	14	116.20 ± 0.14 ^{Bb}	117.55 ± 0.07 ^{Aa}	113.40 ± 0.28 ^{Cb}	117.50 ± 0.14 ^{Ab}
	28	115.53 ± 0.04 ^{Bb}	115.78 ± 1.73 ^{Ba}	115.15 ± 0.35 ^{Ba}	119.88 ± 0.88 ^{Aa}
WI	1	86.62 ± 0.33 ^{Ba}	88.03 ± 0.29 ^{Aa}	87.88 ± 0.76 ^{ABa}	87.14 ± 0.23 ^{ABa}
	14	85.19 ± 0.03 ^{Bb}	86.98 ± 0.06 ^{Ab}	84.77 ± 0.12 ^{Cb}	85.04 ± 0.13 ^{Bb}
	28	84.68 ± 0.15 ^{Bb}	87.71 ± 0.12 ^{Aa}	87.94 ± 0.95 ^{Aa}	81.52 ± 0.018 ^{Cc}

The data were presented as the mean ± standard deviation from three replicate analyses. A-C and a-c present the statistical significance ($p < 0.05$) between the results in the same line and column, respectively. CK: Cow milk kefir, BK: Buffalo milk kefir, EK: Ewe milk kefir, GK: Goat milk kefir

Table 4 Viscosity and rheological properties of the Kefir samples

Parameters	Storage (days)	CK	BK	EK	GK
Viscosity at 50 rpm (cP)	1	1114.29±24.81 ^{Cb}	2756.04±85.79 ^{Aa}	1649.54±30.46 ^{Bb}	316.33±11.48 ^{Db}
	14	1389.56±66.31 ^{Ba}	856.22±36.77 ^{Cb}	2115.11±7.54 ^{Aa}	430.11±0.68 ^{Da}
	28	951.39±11.52 ^{Bc}	871.18±11.82 ^{Cb}	1559.65±0.83 ^{Ac}	95.45±0.28 ^{Dc}
Viscosity at 100 rpm (cP)	1	718.93±0.74 ^{Cb}	1796.84±19.77 ^{Aa}	1113.99±41.32 ^{Bb}	230.97±0.80 ^{Db}
	14	881.61±10.92 ^{Ba}	642.11±11.47 ^{Cb}	1426.00±21.06 ^{Aa}	294.65±6.08 ^{Da}
	28	589.83±1.25 ^{Cc}	642.00±5.49 ^{Bb}	1025.12±0.75 ^{Ac}	66.89±3.54 ^{Dc}
Consistency coefficient (K; Pa.s ⁿ)	1	14.35±0.86 ^{Cb}	51.89±1.84 ^{Aa}	17.98±1.39 ^{Bc}	1.76±0.00 ^{Db}
	14	22.11±0.29 ^{Ca}	49.80±2.29 ^{Ab}	28.05±0.08 ^{Ba}	3.71±0.11 ^{Da}
	28	13.91±0.44 ^{Bb}	9.75±0.13 ^{Cc}	22.36±0.06 ^{Ab}	0.66±0.06 ^{Dc}
Flow behavior index (<i>n</i>)	1	0.34±0.02 ^{Ca}	0.26±0.01 ^{Db}	0.39±0.02 ^{Ba}	0.56±0.01 ^{Aa}
	14	0.30±0.00 ^{Cb}	0.02±0.01 ^{Dc}	0.35±0.00 ^{Bb}	0.45±0.01 ^{Ab}
	28	0.31±0.01 ^{Cab}	0.39±0.00 ^{Ba}	0.32±0.00 ^{Cb}	0.50±0.03 ^{Ab}
R ²	1	0.993±0.002	0.991±0.002	0.998±0.003	0.983±0.007
	14	0.996±0.003	0.979±0.004	0.999±0.000	0.997±0.004
	28	0.997±0.001	0.977±0.002	0.998±0.001	0.977±0.009

The data were presented as the mean±standard deviation from three replicate analyses. A-C and a-c present the statistical significance ($p<0.05$) between the results in the same line and column, respectively. CK: Cow milk kefir, BK: Buffalo milk kefir, EK: Ewe milk kefir, GK: Goat milk kefir

pseudoplastic and the values were between 0 and 1, similar to those reported in published studies [40, 41].

Many factors can affect viscosity and K values, such as total solids content, heat treatment, starter culture type, microorganism content, storage conditions, and the denaturation ratio of whey protein [17]. The differences in this study might have been caused by the different milks used.

Volatile component profile

Composition of volatile compounds of Kefir samples

The compositions of the volatile compounds identified by SPME GC-MS of the kefir samples are given in Table 5. Similarly, many of these flavor compounds have been identified in previous studies of ice cream produced from different animal milks [5], and this similarity is reportedly due to the type of milk. Flavor compounds detected in milk could be related to various biochemical processes, environmental factors, microbial contamination, animal nutrition, or substances used during processing. Furthermore, depending on the type and quantity of compounds, valuable information about the quality, freshness, and processing processes of milk can be obtained [42, 43]. Compounds, such as acetic acid, butanoic acid, hexanoic acid, octanoic acid, benzoic acid, n-decanoic acid, hexadecanoic acid, and dodecanoic acid, which might be produced from natural biochemical processes, can occur as a result of microbial fermentation or degradation of fats in milk [44, 45]. On the other hand, alcohols such as n-butanol, 2-butanol, 2-pentanol, 2-hexanol, 1-hexanol, and 2-nonanol are produced as a result of glycolysis, proteolysis, and lipolysis during fermentation and yeasts are mainly responsible for alcohol production in kefir [44, 46]. Specifically, 2-nonanone, 2-decenal, nonanoic acid, 2-undecanone, and 2-undecanol can be produced because of

lipid oxidation in dairy products [47], and these compounds are likely to increase especially in dairy products exposed to oxygen. Hexanal, decanal, and nonanal aldehydes can be formed from lipid oxidation and amino acid degradation [45, 46]. In addition, terpenes, such as limonene, eucalyptol, α -pinene, p-cymene, (+)-3-carene, α -copaene, caryophyllene, (E)- β -farnesene, cubenene, and 7-epi- α -selinene are generally plant-derived compounds and can pass into milk through the diet [48], although limonene and α -pinene can also be synthesized by microorganisms [49]. Hydrocarbon compounds including dodecane, tridecane, tetradecane, 1-dodecene, 1-hexadecene, and 8-heptadecene are formed from animal feed or as secondary products of lipid oxidation [50]. Conversely, ester compounds (i.e., methyl esters, 1-methylethyl ester, 1,2-benzenedicarboxylic acid, and diethyl ester) are synthesized from fatty acids or alcohols by microbial esterase enzymes [46, 51]. Levomenthol is a compound with biological activity that is usually found in mint [52] and its detection in milk might be the result of feeding animals with plants containing mint or if animals come into contact with such compounds during processing. Finally, p-cresol is an important alkylphenol that is related to the barnyard flavor and might also contribute to undesirable flavors [42].

Agglomerative hierarchical clustering and principal component analysis on volatile compounds of Kefir samples

The agglomerative hierarchical clustering (AHC) results of the volatile compounds are given in Fig. 3. To decide how the clusters were separated, a cutoff point on the dendrogram was determined as the point where the distance increase in the vertical axis was abrupt and this cutoff point gave rise to clusters, a group in which the elements are highly similar to

Table 5 Composition of volatile compounds identified by SPME GC-MS of the Kefir samples

RT ^a (min.)	Compound	Kovats Index	Kovats Index ^b	Concentration (µg/mL)				CK1	CK14	CK28	BK1	BK14	BK28	EK1	EK14	EK28	GK1	GK14	GK28
2.845	Acetic acid	601	601	1.08	3.00	2.87	2.34	2.47	3.39	3.06	3.22	2.84	2.90	3.31	3.31	2.92	2.56	3.44	1.75
3.816	n-Butanol	621	621	10.0***	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4.254	2-Butanone, 3-hydroxy-	662	662	nd	12.21	nd	1.98	9.88	nd	4.44	2.74	10.31	3.23	3.56	5.21	6.72	1.76	6.65	1.12
5.742	2-Butanol, 3-methyl-	671	671	nd	nd	nd	0.48	2.49	8.55	1.31	4.52	9.05	4.97	1.05	1.93	2.65	2.30	9.17	nd
6.164	2-Pentanol	682	682	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.35	nd	2.49
7.798	2-Hexanol	787	787	3.22	2.26	nd	0.79	0.34	1.60	nd	2.15	9.89	2.01	2.12	1.51	7.25	4.17	9.75	14.80
7.992	Butanoic acid	790	790	1.23	0.97	0.97	0.99	1.11	0.87	1.13	0.89	1.10	0.99	1.23	1.13	1.13	0.89	0.89	1.10
8.248	Hexenal	799	801	nd	nd	nd	0.76	0.21	1.08	1.11	3.18	nd	2.87	1.06	nd	0.40	nd	nd	nd
8.897	α-Pinene	913	913	nd	nd	27.14	nd	nd	nd	0.52	nd	nd	nd	1.38	1.11	nd	nd	nd	nd
9.113	1,6-Octadiene, 3,7-dimethyl-	922	922	nd	nd	1.24	nd	nd	nd	nd	nd	nd	nd	2.42	1.57	nd	nd	nd	nd
9.531	Benzaldehyde	948	948	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.62	nd
9.550	2-Octene, 3,7-dimethyl-, (Z)-	958	958	nd	nd	3.45	nd	nd	nd	nd	nd	nd	nd	2.17	2.01	nd	nd	1.69	nd
9.960	Hexanoic acid	992	992	nd	34.47	3.31	10.89	23.29	12.49	nd	15.7	18.31	nd	1.74	2.01	3.68	8.67	0.49	18.82
10.201	2-Menthene, cis	1010	1010	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.44	0.54	0.26	nd	nd	nd	nd
10.414	(+)-3-Carene	1020	1020	nd	nd	1.00	nd	nd	nd	nd	nd	nd	nd	0.42	0.34	nd	nd	nd	0.47
10.654	p-Cymene	1033	1035	nd	nd	4.05	nd	nd	nd	nd	nd	nd	nd	2.06	0.18	nd	nd	nd	nd
10.712	1-Hexanol, 2-ethyl-	1036	1036	1.80	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10.739	Limonene	1038	1038	nd	nd	nd	0.66	nd	nd	0.82	2.23	nd	nd	nd	nd	nd	nd	nd	nd
10.805	Eucalyptol	1041	1041	nd	nd	1.86	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.58	nd	nd
11.481	Heptanoic acid	1077	1077	nd	nd	nd	nd	0.08	nd	nd	nd	1.46	nd	nd	nd	nd	nd	nd	nd
11.516	p-Cresol	1079	1079	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.47	nd
11.787	2-Nonanone	1094	1094	nd	nd	15.25	nd	0.82	1.04	0.52	3.48	0.93	0.76	0.51	0.31	nd	nd	0.99	0.45
11.911	Undecane	1100	1100	nd	nd	nd	2.82	nd	nd	6.16	nd	1.21	6.61	21.68	17.74	7.22	0.33	nd	nd
11.925	2-Nonanol	1101	1101	nd	nd	nd	nd	nd	nd	nd	2.83	nd	nd	nd	nd	0.80	5.80	0.63	0.59
12.009	Nonanal	1107	1107	nd	nd	nd	0.31	nd	0.77	nd	3.95	1.77	0.77	0.49	0.65	nd	nd	0.63	nd
12.911	2-Nonenal, (E)-	1164	1164	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.74	0.34	0.65	nd	0.80	nd	nd
13.195	Levomenthol	1182	1182	nd	nd	nd	29.4	nd	nd	28.43	nd	nd	29.54	nd	nd	19.32	nd	nd	nd
13.280	Octanoic acid	1181	1185	33.00	nd	6.83	13.51	nd	nd	nd	nd	8.23	nd	nd	nd	nd	25.86	nd	nd
13.360	Ethanol, 1-(2-butoxyethoxy)-	1191	1191	nd	nd	nd	nd	nd	nd	nd	nd	6.57	nd	nd	nd	nd	nd	nd	nd
13.360	1-Dodecene	1192	1192	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.87	nd	nd	nd	nd	nd	nd
13.479	Dodecane	1200	1200	nd	2.78	1.00	nd	nd	nd	7.03	1.78	1.87	7.38	nd	nd	nd	nd	nd	nd
13.488	Benzoic acid	1210	1210	nd	23.15	nd	4.74	36.82	22.24	15.92	28.51	5.34	2.82	36.98	39.70	24.48	5.04	11.64	37.65
13.595	Decanal	1208	1208	nd	nd	nd	nd	nd	nd	nd	nd	0.20	0.22	nd	nd	nd	20.01	nd	nd
13.697	Undecane, 5,6-dimethyl-	1215	1215	nd	nd	nd	nd	nd	nd	nd	nd	5.74	0.72	nd	nd	nd	nd	nd	nd
13.877	p-Chavicol	1227	1227	nd	nd	nd	nd	nd	1.92	nd	nd	nd	nd	nd	nd	nd	0.66	nd	nd
14.443	2-Decenal	1261	1261	nd	nd	nd	nd	0.42	0.87	nd	nd	nd	nd	nd	0.19	nd	nd	0.71	nd
14.492	Nonanoic acid	1269	1269	1.81	nd	nd	nd	0.49	nd	0.72	nd	nd	nd	0.16	nd	nd	nd	nd	nd
14.794	3-Tridecene, (E)-	1283	1283	nd	nd	nd	nd	nd	0.26	nd	nd	nd	0.35	nd	0.18	nd	nd	nd	nd
14.879	2-Undecanone	1289	1289	nd	0.71	nd	nd	0.96	1.48	0.51	2.64	nd	0.91	0.35	0.25	0.65	nd	0.86	0.42

Table 5 (continued)

RT* (min.)	Compound	Kovats Index	Kovats Index**	Concentration (µg/mL)															
				CM	BM	EM	GM	CK1	CK14	CK28	BK1	BK14	BK28	EK1	EK14	EK28	GK1	GK14	GK28
14.932	Tridecane	1300	1300	nd	nd	1.57	1.61	nd	nd	3.91	nd	nd	4.94	nd	0.65	2.53	0.39	nd	nd
14.972	2-Undecanol	1303	1303	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.31	0.43	nd	2.43	nd	nd
15.074	β-Methylnaphthalene	1310	1310	0.89	nd	0.82	nd	nd	nd	1.27	2.13	nd	nd	nd	nd	nd	nd	nd	nd
15.785	n-Caprylic acid isobutyl ester	1369	1369	0.34	nd	nd	2.14	nd	0.36	nd	nd	nd	nd	0.34	0.24	0.20	nd	nd	nd
15.869	n-Decanoic acid	1369	1369	21.98	4.16	3.90	15.3	10.84	16.77	9.29	nd	1.92	2.75	3.26	3.53	1.85	nd	34.87	12.94
15.971	Dodecane, 2,6,10-trimethyl-	1377	1378	nd	nd	nd	nd	nd	nd	nd	nd	0.81	nd	nd	nd	nd	9.44	nd	nd
16.176	α-Copaene	1392	1392	0.20	0.94	nd	nd	nd	nd	nd	0.62	nd	nd	0.45	0.47	2.35	nd	1.54	1.12
16.185	4-Tetradecene	1393	1396	nd	nd	nd	1.78	nd	1.13	4.22	nd	nd	5.00	nd	nd	nd	nd	nd	1.33
16.280	Tetradecane	1400	1400	nd	3.10	3.18	1.81	1.53	1.99	nd	2.8	0.16	3.28	nd	0.59	1.32	0.26	nd	nd
16.402	1,5,9-Decatriene, 2,3,5,8-tetramethyl-	1410	1410	nd	nd	4.77	nd	nd	nd	2.72	nd	nd	nd	nd	2.81	0.15	1.60	nd	nd
16.798	Caryophyllene	1441	1441	nd	1.11	4.31	nd	nd	1.01	nd	1.07	nd	nd	nd	1.82	1.46	nd	2.23	1.64
16.900	(E)-β-Farnesene	1449	1449	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.16	0.17	0.19	1.08	nd	nd
17.074	2,6,10-Trimethyltridecane	1463	1463	0.76	0.87	1.36	nd	nd	0.84	nd	0.73	0.64	0.69	0.51	0.50	0.70	nd	nd	nd
17.251	1-Dodecanol	1484	1485	0.71	nd	nd	nd	nd	2.19	nd	nd	nd	nd	1.58	2.30	0.48	nd	nd	nd
17.544	pentadecane	1499	1499	1.92	1.62	nd	4.91	nd	1.32	1.42	2.82	nd	2.04	nd	0.31	0.53	0.47	1.36	0.30
17.597	β-Bisabolene	1503	1503	nd	nd	2.19	nd	1.03	4.03	nd	nd	nd	1.69	1.61	0.63	0.53	0.85	0.65	nd
17.649	δ-Amylvalerolactone	1508	1508	1.99	2.09	0.95	nd	nd	3.04	1.39	3.26	2.56	1.69	nd	0.51	1.52	nd	1.60	nd
17.789	γ-Murolene	1515	1513	nd	nd	nd	nd	1.7	nd	nd	nd	nd	nd	0.51	nd	nd	0.43	nd	nd
18.024	σ-Cadinene	1540	1540	nd	nd	3.98	nd	nd	nd	nd	nd	0.32	nd	0.51	1.38	nd	nd	nd	0.63
18.061	cis-Calamenene	1543	1543	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.23	nd	nd	0.29	0.39	nd
18.145	Cubenene	1557	1552	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.37	nd	nd	nd	nd	nd
18.241	7-epi-α-Selinene	1557	1557	nd	nd	nd	nd	nd	1.75	nd	nd	0.37	nd	0.61	nd	nd	nd	nd	nd
18.299	Cetene	1585	1585	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd
18.303	n-Dodecanoic Acid	1563	1580	5.11	nd	nd	0.9	nd	nd	1.5	nd	nd	nd	nd	nd	nd	nd	0.82	nd
18.641	1-Hexadecene	1592	1592	2.03	nd	nd	nd	0.18	nd	nd	nd	nd	0.33	nd	nd	nd	nd	nd	nd

Table 6 Performance parameters of the method in HPLC for benzoic acid analysis (Aktaş et al. 2024)

Performance parameters	Results
Linear range (µg/mL)	0.1–250
Calibration curve equation	$y = 97.63445x + 24.88712$
R ²	0.99993
LOD (µg/mL)	0.330
LOQ (µg/mL)	1.10
% Recovery for 5 µg/mL	101
% Recovery for 500 µg/mL	98.5

R²: square of regression coefficient; LOD: limit of detection; LOQ: limit of quantification

Comparison of different animal milks on microbiological, physicochemical, sensory properties, and volatile component profile of kefir

each other. These groups describe similar characteristics or patterns in terms of volatile compounds and the size of the clusters and the number of elements in them also represent the number of clusters. For example, large clusters contain many similar data points, whereas small clusters represent more specialized groups.

In the dendrogram, four clusters were formed: cow milk (CM) and GK-1 (i.e., GK on day 1) in cluster 1; buffalo milk (BM), CK-1, CK-14, BK-1, BK-14, EK-1, EK-14, EK-28, GK-14, and GK-28 in cluster 2; ewe milk (EM) in cluster 3; and goat milk (GM), CK-28, and BK-28 in cluster 4. In particular, the number of elements in the clusters provides

important information about the structural similarities of the data. For example, the presence of more elements in cluster 2 indicates a strong similarity between these elements, whereas the EM element alone implies different characteristics compared with the others. In cluster 3, the amount of α -pinene present in the EM was 27.14 µg/mL, whereas it was 0.52, 1.38, and 1.11 µg/mL in CK-28, EK-1, and EK-14, respectively. Furthermore, in cluster 1, octanoic acid was detected at 33.00 µg/mL in CM, 25.86 µg/mL in GK-1, 6.83 µg/mL in EM, 13.51 µg/mL in GM, and 8.23 µg/mL in BK. In cluster 4, levomenthol was detected at 29.4 µg/mL in GM, 28.43 µg/mL in CK-28, and 29.54 µg/mL in BK-28, whereas it was 19.32 µg/mL in EK-28. Levomenthol was not detected in the other samples.

The cluster structure and compound distribution significantly differ between the chemical contents of the samples. In particular, the high amount of α -pinene in EM was significantly different from that in the other samples. Likewise, the presence of levomenthol in the samples in cluster 4 suggests that the characteristic properties of these samples are different. The chemical profiles of CM and GK-1 are significantly different from those of the other samples, indicating that octanoic acid might be a distinctive compound in these clusters.

The principal component analysis (PCA) results are presented in Fig. 4. The PCA plot shows a clear separation

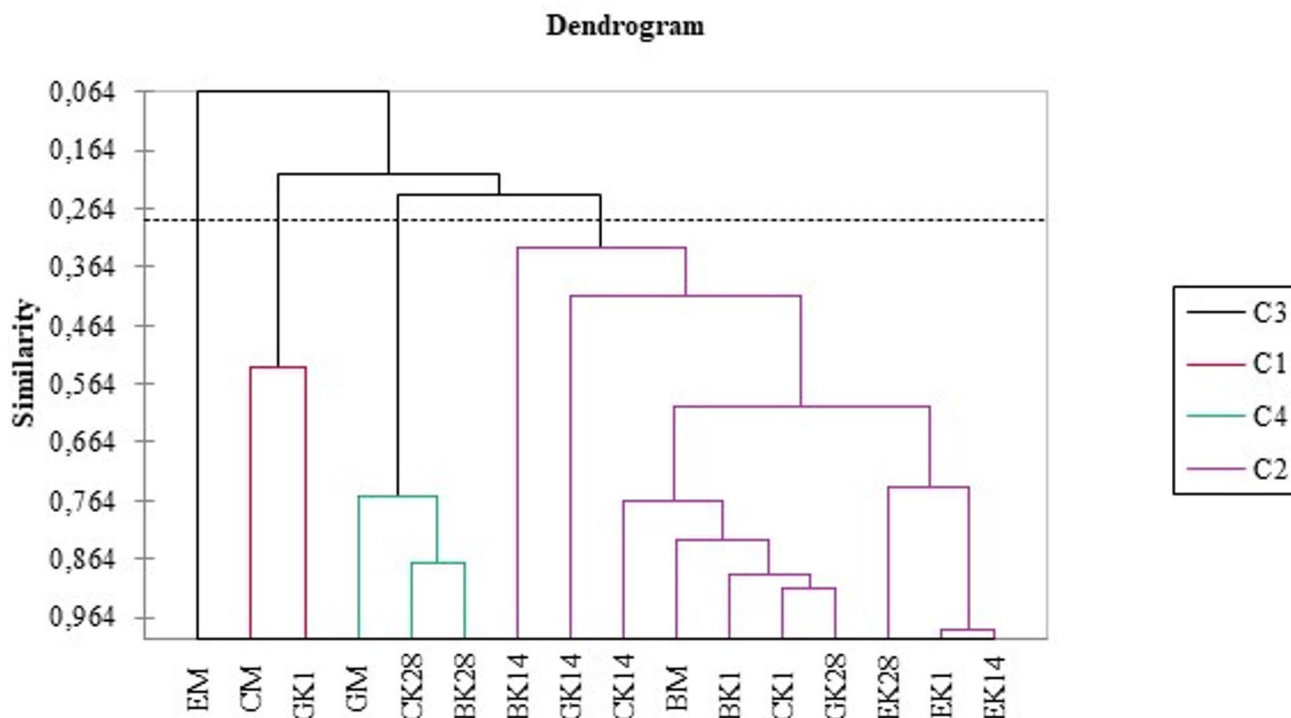


Fig. 3 AHC analysis results on the volatile compounds of the kefir samples. CM: Cow milk, BM: Buffalo milk, EM: Ewe milk, GM: Goat milk, CK: Kefir produced using cow milk, BK: Kefir produced using

buffalo milk, EK: Kefir produced using ewe milk, GK: Kefir produced using goat milk

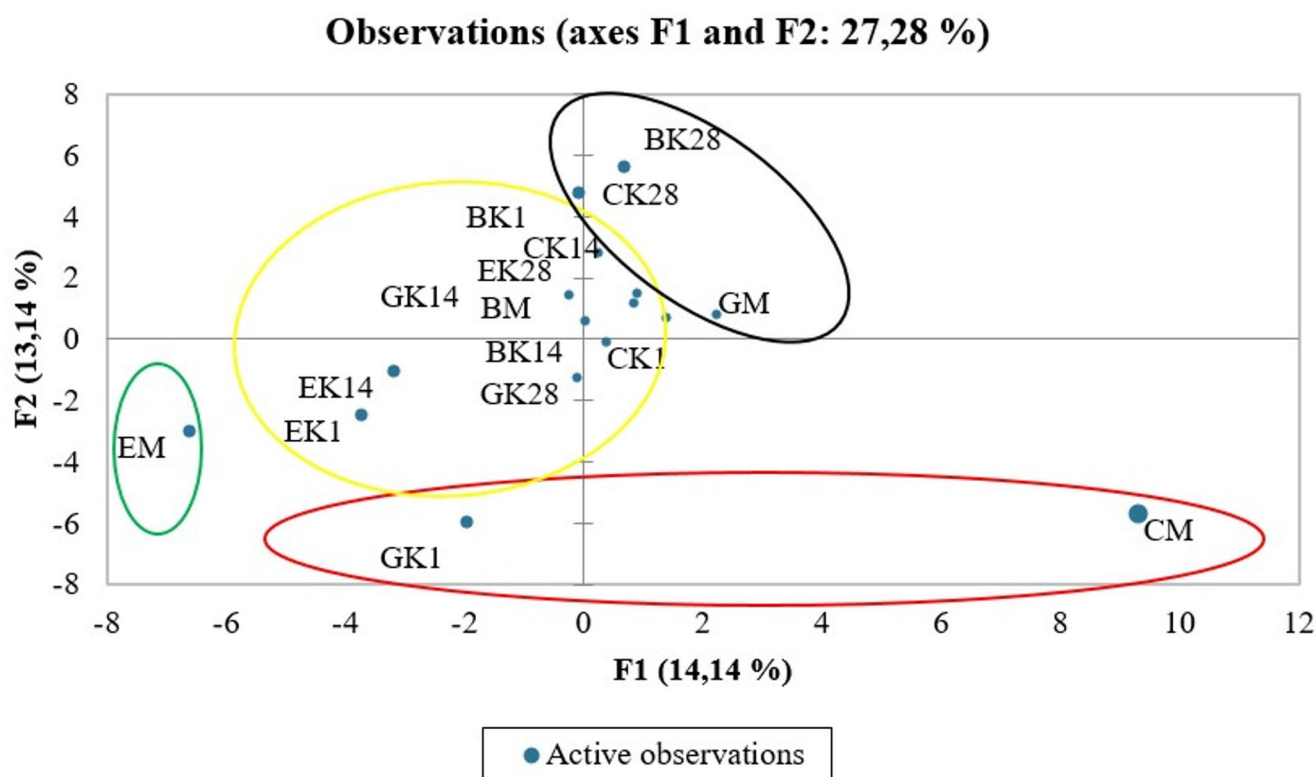


Fig. 4 PCA analysis results on the volatile compounds of the kefir samples. CM: Cow milk, BM: Buffalo milk, EM: Ewe milk, GM: Goat milk, CK: Kefir produced using cow milk, BK: Kefir produced using

buffalo milk, EK: Kefir produced using ewe milk, GK: Kefir produced using goat milk

among kefir samples based on milk type, with each sample group forming distinct clusters. This separation is consistent with the AHC results, supporting the reliability of the clustering structure. The alignment of both multivariate methods highlights the distinct volatile profiles of kefir produced from cow, buffalo, ewe, and goat milk, emphasizing the impact of milk origin on product characteristics. In the PCA plot, CM and GK-1 were in the same cluster, indicating that the chemical profiles of these two samples are similar to those in the AHC results. The presence of more samples in cluster 2 indicates that the structural similarities between these samples are strong and the that group has common features. The close positioning of these samples in the PCA confirms that they have common chemical properties and that the clustering is meaningful. The EM sample was placed alone in cluster 3 and was different from the other samples in both the AHC and PCA analyses. This finding indicates that the chemical profile of EM is markedly different from that of the other samples and has unique characteristics. In particular, the high abundance of compounds, such as α -pinene in the EM sample, might be an important component causing this difference. The fact that GM, CK-28, and BK-28 are in the same cluster according to both the PCA and AHC analysis suggests that the chemical contents

of these samples are similar. The presence of levomenthol in this cluster caused the characteristic properties of these samples to differ.

Benzoic acid content

The benzoic acid contents of the kefir samples during different storage periods are shown in Fig. 5 and Table S1. The performance parameters of the HPLC method for benzoic acid analysis are given in Table 6. The benzoic acid content in the kefir samples did not significantly change throughout the storage duration ($p > 0.05$). On the other hand, the order from the highest to lowest-benzoic acid content in the samples was EK, GK, CK, and BK during the whole storage period, and these differences were statistically significant ($p < 0.05$). The results revealed that benzoic acid in fermented foods could be produced from hippuric acid by LAB during fermentation, as also reported by Aktaş et al. [17]. There are few studies on the benzoic acid content in kefirs; therefore, this study is one of the first to report on this subject. In the study by Şendoğan et al. [53], the benzoic acid contents in kefir made from goat milk was between 9.5 and 25.1 $\mu\text{g/mL}$ during storage. Moreover, similar to this study, others have reported that kefirs [17] and other

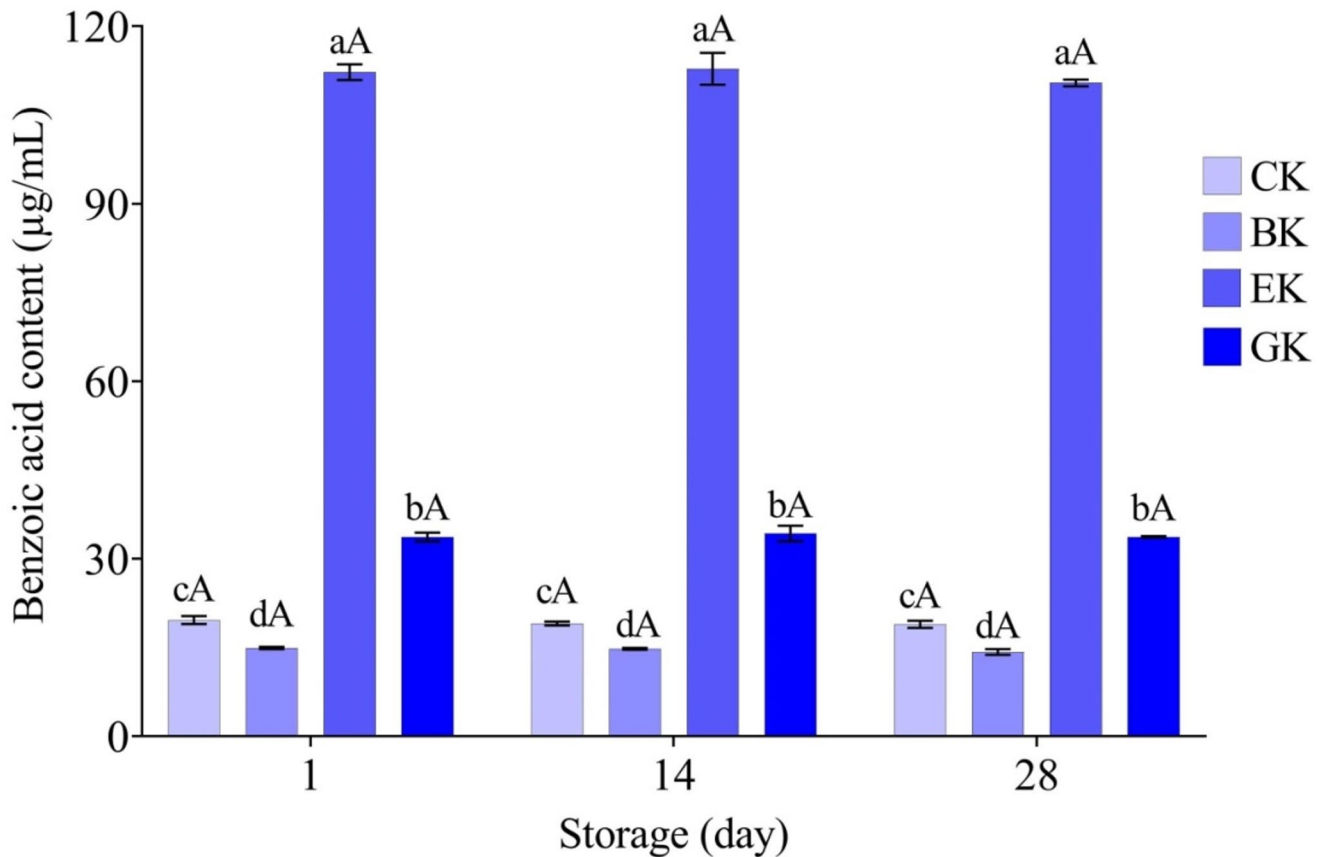


Fig. 5 Benzoic acid contents of kefir samples during storage (The data were presented as the mean $\pm$ standard deviation from three replicate analyses. **a-d** indicates statistical differences in the same day. **A-C** indicates statistical differences at different days of same sample. CK:

Kefir manufactured using cow milk BK: Kefir manufactured using buffalo milk EK: Kefir manufactured using sheep milk. GK: Kefir manufactured using goat milk)

fermented products [54, 55] produced from ewe milk had higher benzoic acid contents than cow, buffalo, and goat milk. This difference is believed to be due to the higher hippuric acid content of ewe milk than of other milk [54].

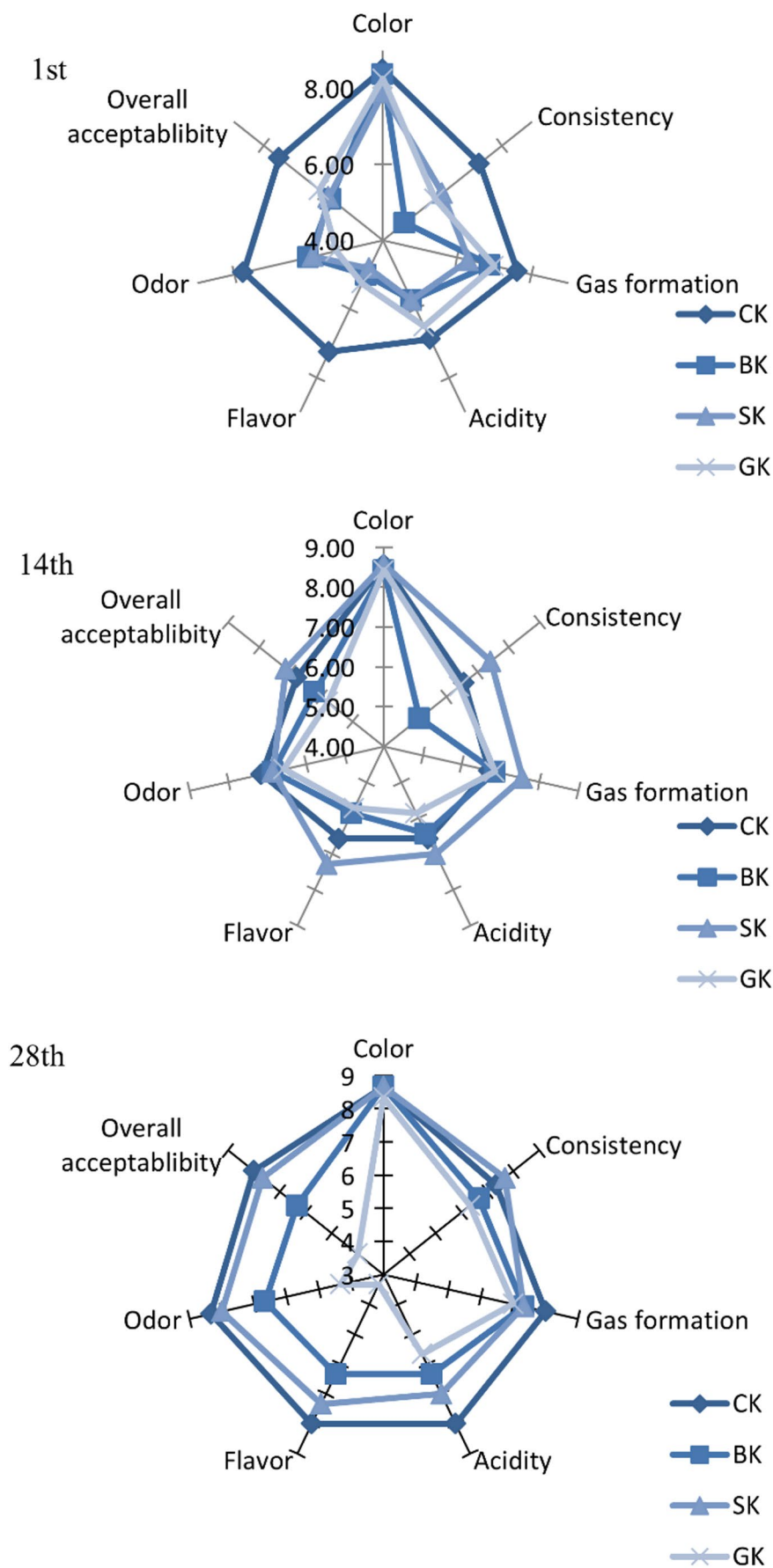
Sensory properties

The CK sample was more appreciated by the panelists than other the kefirs were on day 1 in terms of overall sensory properties (Fig. 6). The color scores were very similar among the samples throughout the storage period. On day 14 of storage, EK had the highest scores for all the sensory properties except color and odor. All scores for CK were the highest on day 28 except for color. GK had the lowest flavor and overall acceptability scores during storage, except on day 1 (Fig. 6). Similar results have been reported in previous studies [17, 37–39].

Conclusion

The properties of kefirs differ from each other because of the variability of the components in milk from different animals. This difference was also revealed in the aroma profiles of the samples and four different clusters were identified via both PCA and AHC analyses. According to the sensory analysis results, CK and EK generally presented the highest scores on different days of storage. The data obtained from this study show that in addition to CM, BM and sheep milk kefir can be produced commercially and offered as alternative products to consumers. These products may appeal to niche markets seeking novel flavors, traditional dairy alternatives, or functional foods with unique microbial profiles. Future studies should investigate the specific health-promoting properties of kefirs produced from different types of animal milk, as well as consumer acceptance and market feasibility on a larger scale.

Fig. 6 Sensory properties of kefir samples overall during storage (1st, 14th, and 28th days) (CK: Kefir manufactured using cow milk BK: Kefir manufactured using buffalo milk SK: Kefir manufactured using sheep milk. GK: Kefir manufactured using goat milk)



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Declarations

Present study was approved by the Gümüşhane University Research Ethics Committee (2022/6 and 26/10/2022). All panellists were informed about the study and written informed consent was obtained prior to sensory analysis.

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