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Comparative study of the aroma profiles and chemical and microbiological properties of bovine kefir enriched with lyophilized colostrum of different milk kinds

Tarık Yörükoğlu[2], Melike Demirkol[1], Ali Cingöz[1]✉, Zeynep Yaren Pehlivan[3] & Serkan Selli[3]

In this study, the effects of lyophilized colostrum powders (1%; cow, buffalo, sheep, and goat) on the aroma profiles and physicochemical and microbiological properties of kefir were investigated. The colostrum supplement maintained pH stability and improved water holding capacity and synergy. This also limited the decline in color parameters. According to the microbiological results, Lactococci counts (7.96–8.63 log CFU/mL) remained stable throughout storage. Lactobacilli counts markedly decreased in the control group, whereas all colostrum-supplemented samples maintained values above 7 log CFU/mL. A total of 34 aroma compounds were identified in the produced kefir samples, and their distribution significantly differed depending on the animal from which the colostrum was obtained ($p < 0.05$). The basic aroma classes included alcohols, ketones, acids, and aldehydes. The richest aroma profiles were found in kefir enriched with sheep, cow, goat, and buffalo colostrum, respectively. Acids (acetic, butanoic, and hexanoic acids) were the most prevalent compound group in all the samples. More alcohol varieties were detected in kefir made with sheep and cow colostrum. In conclusion, adding 1% colostrum powder from various animal species has a positive effect on the physical stability and microbial balance of kefir.

Keywords Buffalo, Functional dairy products, GC–MS, Goat, Lactic acid bacteria, Volatile compounds

Various fermented milk products are produced commercially worldwide. One such product is kefir[1]. Derived from the Turkish word 'kef', meaning 'good feeling' or 'health', kefir is a low-alcohol, acidic, fermented beverage produced by adding kefir grains to milk or a sucrose solution[2]. It is produced and consumed commercially in many countries, including, Europe, Asia, South America, and North America[3]. Production of kefir is divided into three categories based on the raw material used: milk kefir; kefir-like products; and plant-based products[4–10]. Kefir is classified as a functional beverage and has antimicrobial[11], anti-inflammatory[12], antiallergic, cholesterol-lowering[13], and antioxidant[14] properties. These health benefits are attributed to the unique microbiota of kefir and the bioactive compounds produced during fermentation[15]. Microbial interactions between yeast and bacteria during kefir fermentation affect the flavor, quality, and aroma of the final product[16]. The aroma of traditional kefir is influenced by the microbiological composition of the kefir grains used in production, the metabolic activity of bacterial and yeast species, symbiotic interactions between microorganisms[17], production methods, and the type and composition of the raw material[16].

On average, the colostrum of animals such as cows, goats, sheep, pigs and camels contains 17.9–32.8% dry matter, 3.3–21.7% protein, 1.7–10.6% fat, 1.7–4.4% lactose and 0.59–0.77% ash[65,66]. The increasing popularity of nutritious and functional foods among consumers is generating interest in colostrum, which has a rich nutritional composition[18]. Secreted by mammals after birth, colostrum is known as 'liquid gold' and belongs to

[1]Department of Food Engineering, Tokat Gaziosmanpasa University, 60150 Tokat, Turkey. [2]Department of Food Engineering, Kahramanmaraş Sütçü Imam University, 46250 Avşar, Kahramanmaraş, Turkey. [3]Department of Food Engineering, Faculty of Agriculture, Çukurova University, Adana, Turkey. ✉email: ali.cingoz@gop.edu.tr

the nutraceutical food group becasue of its high content of bioactive compounds and essential nutrients[19,20]. Sold in various forms (powder, liquid, and tablet), colostrum is used in the food and pharmaceutical industries[21–24]. To extend its shelf life and enable its use in different industries, it is recommended that liquid colostrum be converted into powder form[25]. Freeze-drying (lyophilization), which causes minimal damage to a product's chemical structure and preserves its nutritional value and aroma, is the preferred method for this purpose. However, a literature review revealed that few studies have investigated the lyophilization of colostrum[28,29].

Aroma is one of the most important quality parameters that significantly influences consumer acceptance of and preference for food products. These volatile compounds often change, deteriorate, and/or disappear during food processing. They play a key role in determining consumer acceptance because they affect the quality of the final product[30]. Hundreds of aroma compounds have been identified in kefir[9,16]. In generally, milk kefirs contain a more diverse range of aroma compounds than water kefirs do. The predominant groups of volatile compounds in kefirs are acids, ketones, alcohols, aldehydes, and esters[3]. The taste and aroma of kefir are influenced by organic acid components such as formic acid, succinic acid, propionic acid, and lactic acid, as well as acetaldehyde, diacetyl, acetone, and ethanol[31]. As a result of fermentation involving lactic acid bacteria and yeasts, kefir contains lactic acid, acetic acid, and trace amounts of carbon dioxide, ethanol, and aromatic compounds. Consequently, kefir acquires a distinctive aroma with pronounced organoleptic properties not found in other fermented milk products[1].

Studies evaluating the microbial diversity and aroma components of kefir prepared using different methods can be found in the literature. However, detailed aroma compositions and microbial characterisations of kefir beverages made enriched with cow, sheep, goat, and buffalo colostrum have not yet been reported. This study investigated the effects of 1% lyophilised colostrum powders (cow, buffalo, sheep and goat) on the physicochemical, color and microbiological properties of kefir. Additionally, a comprehensive characterization of the aroma profiles of these colostrum-enriched milk kefir samples was conducted.

Materıal and methods

Materials

Colostrum samples were obtained from Karayaka sheep, Saanen goats (*Capra aegagrus hircus*), Danish Red cattle and crossbred buffalo (*Bubalus bubalis*) from local farms in the Kahramanmaraş and Tokat provinces of Turkey. The methods used to obtain the colostrum samples and perform detailed composition analyses were previously reported by Cingöz & Yörükoğlu[32]. The moisture content of freeze-dried cow, sheep, buffalo, and goat colostrum is 4.78%, 5.50%, 3.92%, and 4.36%, respectively, the fat content is 16.69%, 35.78%, 40.21%, and 29.20%, protein content is 34.87%, 38.07%, 42.43%, and 28.29%, and ash content is 0.95%, 1.03%, 0.98%, and 0.66%. Commercial pasteurized cow milk (Mis Süt, Ak Gida San. ve Tic. A.Ş., Sakarya, Turkey; 4.7% carbohydrates, 3.1% fat, 2.8% protein) and a commercial kefir starter culture (CHR Hansen, Denmark) were used to produce the kefir. All reagents were obtained from Sigma Chemical Company (MO, USA), Merck KGaA (Darmstadt, Germany), or Alfa Aesar GmbH & Co. KG (Germany). Microbiological media and sterile saline solution (0.85% NaCl) used for serial dilutions were supplied by Merck (Darmstadt, Germany).

Methods

Freeze-drying

Liquid colostrum samples were transferred to glass drying containers, frozen at -80 °C for six hours, and then freeze-dried under vacuum (0.011 mbar) at − 75 °C for 48 h (Christ Gamma, Alpha 2–4 LO + , Germany). The dried powder was collected when the moisture content was < 5%, packaged in a dark glass bottle, and stored at − 18 °C until analysis.

Kefir production

Kefir was produced according to a modified procedure based on previous studies. Cow milk was pasteurized at 85 °C for 20 min and subsequently cooled to 24 °C. After cooling, lyophilized colostrum powders obtained from cows, buffalos, goats, and sheep were individually added at a concentration of 1% (w/v). A commercial DVS kefir starter culture, eXact Kefir 1 (CHR Hansen, Denmark), was subsequently inoculated into the milk mixture. This starter culture is a blend of mesophilic aromatic lactic acid bacteria (type LD), a thermophilic culture, and yeast, and includes *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Streptococcus thermophilus*, *Leuconostoc* spp., and *Debaryomyces hansenii*. Fermentation was carried out at 24 °C for 17 h. At the end of the fermentation period, kefir samples were cooled and stored at 4 °C for 14 days. Physicochemical, microbiological, and aroma profile analyses were performed on days 1, 7, and 14 of refrigerated storage. Kefir production was carried out three times. All analyses were performed in triplicate. Microbiological and physicochemical analyses were conducted on the designated storage days, while aroma profile and total solids analyses were performed on the first day of storage.

Total solid determination

The total solids content of the kefir samples was determined using the gravimetric method[33]. Approximately 10 g of each sample was dried in a hot air oven at 105 °C until a constant weight was reached. The measurements were performed in triplicate, and the results are expressed as a percentages (%).

pH and titratable acidity

The acidity of kefir was determined by measuring the pH and titratable acidity (% lactic acid). For the pH measurement, approximately 20 mL of kefir was placed in a beaker, and a digital pH meter (Hanna, HI 2211, Woonsocket, USA) electrode was inserted until a stable reading was obtained. For titratable acidity, 10 mL of kefir was titrated with 0.1 N NaOH, and phenolphthalein was used as an indicator. The volume of NaOH

consumed was used to calculate the acidity, expressed as % lactic acid. All measurements were performed in triplicate.

Syneresis and water holding capacity (WHC)
Syneresis of the kefir samples was assessed following the procedure described by Bensmira and Jiang[34], with minor adjustments to fit the experimental conditions of this study. A total of 10 g of kefir was placed in a centrifuge tube and centrifuged at 6000 rpm for 15 min at 4 °C.

Syneresis (%) was calculated as folows: Syneresis (%) = B × 100 \ C
where B is the weight of the whey after centrifugation and C is the initial sample weight.
Water holding capacity (%) was then calculated as folows: WHC (%) = 100 – Syneresis (%)
All measurements were performed in triplicate.

Color measurement
The color properties of the kefir samples were determined using a Minolta Chroma Meter (CR-300, Osaka, Japan). Each sample was transferred directly into the measurement cell of the instrument, ensuring that the surface was free of air bubbles and evenly distributed. The color parameters L* (lightness), a* (red-green axis), and b* (yellow-blue axis) were recorded. All the measurements were performed at room temperature, and the results are expressed as the means of three replicates.

Microbiological analysis
Microbiological enumeration of kefir samples was performed according to the method described by Atalar[35] to determine the populations of *Lactococci*, *Lactobacilli*, and yeasts. For each analysis, 10 g of kefir was aseptically mixed with 90 mL of sterile saline (0.85% NaCl) to prepare a 1:10 dilution, followed by serial decimal dilutions as needed. Lactococci were enumerated on M17 agar under aerobic conditions at 30°C for 48–72 h, Lactobacilli on de Man, Rogosa and Sharpe (MRS) agar under anaerobic conditions at 30°C for 48–72 h, and yeasts on Yeast Extract Glucose Chloramphenicol (YGC) agar at 25°C for 48–72 h. All colonies were counted, and the results are expressed as log CFU/mL of kefir. Analyses were performed in triplicate to ensure accuracy.

Extraction process for aroma compounds
Aroma compounds were extracted from kefir samples enriched with goat, sheep, buffalo, and cow colostrum (kefir samples cooled to 4 °C at the end of the fermentation period) using purge and trap methods. For this purpose, 6 g of kefir samples were placed in 20 mL glass vials, to which 5 µL of 4-nonanol and 2-methyl-3-heptanone were added as internal standards. After the vial caps were closed, two holes were made for the carrier gas and trap connections. The traps were conditioned with 6 mL of dichloromethane prior to use. The samples were preincubated for 10 min in a water bath at 60 °C before start of the gas flow. Then, nitrogen gas was passed through each vial at a rate of 500 mL/min, and the aroma compounds were trapped in traps containing 200 mg of Lichrolut EN resin. This process was maintained in a 60 °C water bath for 90 min. The traps were then removed and washed with 12 mL of dichloromethane. The samples were then dehydrated with anhydrous sodium sulfate, and the volume of the solution containing the aroma components was reduced to 0.5 mL in a Vigreux distillation column at 50 °C. The resulting concentrated extracts were transferred to insert vials and stored at – 18 °C prior to analysis[36,37].

GC–MS conditions
A Shimadzu Nexis GC-2030 gas chromatograph and Shimadzu GC–MS-QP2020NY mass spectrometer were used to identify and quantify aroma compounds. The column output was split into the FID detector, and to the MS detector. Quantification of the aroma compounds was performed using GC-FID with a DB-WAX capillary column (60 m × 0.25 mm × 0.4 µm). The temperature programme was set to 3 min at 60 °C, followed by increases of 2 °C per min to 220 °C and then 3 °C per min to 245 °C, with a 20-min hold at the final temperature. The injector temperature was set to 220 °C and the detector temperature was set to 250 °C. Two microlitres of the sample were injected, and helium was used as the carrier gas at a flow rate of 1.5 ml/min. The results are reported in µg/kg.

Data analysis
Statistical analysis was performed using Origin 2024 (Origin Lab Corp., USA). Differences among sample means were evaluated using Tukey's test at a significance level of $p < 0.05$. Principal component analysis (PCA) was also performed to explore the relationships among the variables. All the data used for PCA were normalized. All the experiments were conducted in triplicate, and the results are expressed as the means ± standard deviations.

Results and discussion

The total solids (TS) content of kefir samples prepared with different types of colostrum is shown in Table 1. The TS values ranged from 12.65% to 14.61%, with the lowest value recorded in the control sample. All colostrum-supplemented samples exhibited significantly higher ($p < 0.05$) total solids than the control, while no significant differences ($p > 0.05$) were observed among the colostrum-enriched kefirs. This increase was expected, since the addition of 1% lyophilized colostrum powder contributed extra protein, fat, and mineral components, naturally increasing the total solids content. Similar trends have been reported in previous studies where colostrum or other protein-rich ingredients were incorporated into fermented milk products, leading to increased total solids and improved textural properties[38].

During storage, a decrease in pH was observed in all kefir samples (Table 2). By day 14, the control sample reached the lowest pH, while kefirs enriched with sheep, goat, and cow colostrum maintained higher pH values,

Sample	Control	Cow	Goat	Buffalo	Sheep
Total solids (%)	12.65 ± 0.08^{B}	14.52 ± 0.07^{A}	14.57 ± 0.31^{A}	14.61 ± 0.22^{A}	14.40 ± 0.11^{A}

Table 1. Total solids content of cow kefir samples enriched with 1% colostrum of different origin. The values are expressed as the means ± standard deviations (SDs). Different uppercase letters within the same row indicate significant differences between colostrum types ($p < 0.05$). Total solids content was measured once after kefir production ($p < 0.05$).

Parameter	Sample	Storage (day)		
		1	7	14
pH	Control	4.32 ± 0.02^{Ac}	4.32 ± 0.02^{Ac}	4.25 ± 0.01^{Bc}
	Cow	4.37 ± 0.02^{Aa}	4.36 ± 0.00^{Aa}	4.32 ± 0.01^{Ba}
	Goat	4.34 ± 0.00^{Ab}	4.34 ± 0.00^{Ab}	4.30 ± 0.00^{Bb}
	Buffalo	4.36 ± 0.01^{Aa}	4.36 ± 0.00^{Aa}	4.31 ± 0.01^{Ba}
	Sheep	4.34 ± 0.01^{Bb}	4.37 ± 0.01^{Aa}	4.32 ± 0.01^{Ca}
TA %	Control	0.94 ± 0.01^{Ad}	0.91 ± 0.00^{Bc}	0.90 ± 0.00^{Bd}
	Cow	0.97 ± 0.01^{Ac}	0.95 ± 0.02^{Aa}	0.88 ± 0.01^{Be}
	Goat	1.02 ± 0.01^{Ba}	0.94 ± 0.00^{Ca}	1.04 ± 0.01^{Aa}
	Buffalo	1.00 ± 0.00^{Ab}	0.93 ± 0.00^{Bb}	0.93 ± 0.01^{Bc}
	Sheep	1.00 ± 0.01^{Aa}	1.02 ± 0.01^{Aa}	0.96 ± 0.00^{Bb}

Table 2. Changes in pH and titratable acidity (TA, % lactic acid) of cow kefir samples enriched with 1% colostrum of different origin during 14 days of storage. The values are expressed as the means ± standard deviations (SDs). Different uppercase letters within the same row indicate significant differences over storage time ($p < 0.01$), and different lowercase letters within the same column indicate significant differences among kefir types ($p < 0.01$). TA; Titratable acidity (% lactic acid).

with these differences being statistically significant ($p < 0.05$). Regarding total acidity (TA), all samples except the goat colostrum-enriched kefir showed a decrease during storage. In colostrum-enriched samples, this decrease became more pronounced towards the end of storage; however, on day 14, goat, buffalo, and sheep colostrum-enriched kefirs exhibited higher acidity values compared to the control and cow colostrum-enriched samples. These results indicate that the addition of 1% lyophilized colostrum may help maintain a more stable pH in kefir during storage. On day 14, the control sample exhibited the lowest pH, whereas kefirs enriched with sheep, goat, and buffalo colostrum maintained higher pH values. Yeast counts were highest in buffalo and sheep colostrum-enriched kefirs and lowest in the control; however, total acidity in the colostrum-enriched samples generally remained higher than in the control and cow colostrum-enriched kefir. This suggests that while colostrum addition may have promoted yeast activity, acid production occurred in a balanced manner, contributing to a more stable pH and acidity profile throughout storage. Similar trends have been reported in fermented milk products enriched with colostrum or protein-rich ingredients, where the pH decreases moderately over time due to lactic acid production, whereas the overall acidity remains within acceptable ranges[39,40]. Our results align with previous findings that yeasts in kefir contribute to acidity development; however, despite higher yeast counts in buffalo and sheep colostrum-enriched kefirs, total acidity remained generally higher than the control, suggesting that colostrum may have balanced acid production and maintained a more stable acid profile during storage.

The observed moderate decrease in pH and largely stable titratable acidity in colostrum-enriched kefir samples may be linked to the interplay between yeast populations and lactic acid bacteria during fermentation and storage[41]. Yeasts contribute to kefir acidity by metabolizing lactose and producing ethanol, CO_2, and organic acids, and thus may accelerate acidification if highly active[42]. In products supplemented with colostrum, the additional protein, fat and growth-favoring nutrients might alter microbial dynamics, possibly restraining excessive yeast proliferation and thereby moderating acid generation. In one study, although increased yeast counts were associated with increased acidity in milk kefir, the presence of colostrum appeared to stabilize the pH and acid development during storage[38]. Therefore, the more controlled acid profile in our colostrum-supplemented kefirs might reflect a shift in microbial balance: sufficient yeast and LAB activity to maintain fermentation but not excessive enough to drive the pH precipitously downward. This aligns with our finding of no major differences in titratable acidity among treatments despite slight pH shifts, suggesting that yeast-mediated acid production was moderated by colostrum enrichment[43].

As shown in Table 3, the syneresis values of the kefir samples ranged from 28.74 to 49.47%, while the water holding capacity (WHC) ranged from 50.53 to 71.27% during a 14-day storage period. Overall, syneresis decreased over time, indicating improved gel stability, whereas WHC increased, reflecting enhanced water retention. The control samples exhibited the highest initial syneresis and the lowest WHC, while colostrum-supplemented kefirs generally showed lower syneresis and higher WHC throughout storage, with buffalo and goat colostrum samples exhibiting the most pronounced effects. In the sheep colostrum samples, although the changes in syneresis and WHC were not statistically significant ($p > 0.05$), a decreasing tendency in syneresis

Parameter	Sample	Storage (day)		
		1	7	14
Syneresis (%)	Control	49.47 ± 0.55^{Aa}	39.73 ± 1.18^{Ba}	36.13 ± 0.72^{Ba}
	Cow	38.24 ± 0.35^{Ab}	38.01 ± 1.22^{Aab}	33.36 ± 3.33^{Bab}
	Goat	36.85 ± 0.51^{ABb}	39.94 ± 0.70^{Aa}	33.63 ± 1.52^{Bab}
	Buffalo	36.41 ± 0.79^{Ab}	28.74 ± 0.36^{Bb}	30.20 ± 1.82^{Bb}
	Sheep	37.30 ± 0.79^{Ab}	36.61 ± 1.44^{Aab}	35.07 ± 0.17^{Aab}
WHC (%)	Control	50.53 ± 0.55^{Bb}	60.27 ± 1.18^{Ab}	63.87 ± 0.72^{Ab}
	Cow	61.76 ± 0.35^{Ba}	61.99 ± 1.22^{Bab}	66.64 ± 3.33^{Aab}
	Goat	63.15 ± 0.51^{ABa}	60.06 ± 0.70^{Bb}	66.37 ± 1.52^{Aab}
	Buffalo	63.59 ± 0.79^{Ba}	71.27 ± 0.36^{Aa}	69.80 ± 1.82^{Aa}
	Sheep	62.70 ± 0.79^{Aa}	63.39 ± 1.44^{Aa}	64.93 ± 0.17^{Aab}

Table 3. Syneresis (%) and water holding capacity (WHC, %) of cow kefir samples enriched with 1% colostrum of different origin during 14 days of storage. The values are expressed as the means ± standard deviations (SDs). Different uppercase letters within the same row indicate significant differences over storage time ($p < 0.05$), and different lowercase letters within the same column indicate significant differences among kefir types ($p < 0.05$). WHC; Water holding capacity.

Parameter	Sample	Storage (day)		
		1	7	14
L*	Control	70.65 ± 0.59^{Ac}	65.00 ± 0.36^{Bb}	65.33 ± 0.08^{Bb}
	Cow	66.84 ± 0.34^{Ad}	65.98 ± 0.37^{Ab}	61.38 ± 0.09^{Bc}
	Goat	72.67 ± 0.28^{Ab}	67.24 ± 0.82^{Bb}	65.71 ± 0.44^{Cb}
	Buffalo	75.34 ± 0.07^{Aa}	73.56 ± 0.81^{ABa}	72.57 ± 0.74^{Ba}
	Sheep	70.13 ± 0.20^{Ac}	65.68 ± 0.17^{Bb}	65.11 ± 0.68^{Bb}
a*	Control	-1.99 ± 0.02^{Ba}	-1.91 ± 0.04^{Ba}	-1.59 ± 0.07^{Aa}
	Cow	-2.05 ± 0.01^{Bb}	-2.41 ± 0.01^{Bb}	-1.47 ± 0.02^{Aa}
	Goat	-2.28 ± 0.01^{Bc}	-2.53 ± 0.03^{Bb}	-1.49 ± 0.01^{Aa}
	Buffalo	-1.97 ± 0.02^{Aa}	-1.91 ± 0.10^{Aa}	-1.87 ± 0.06^{Ab}
	Sheep	-2.06 ± 0.02^{Bb}	-1.88 ± 0.04^{Aa}	-1.83 ± 0.02^{Ab}
b*	Control	5.54 ± 0.04^{Ad}	5.22 ± 0.11^{Bb}	4.96 ± 0.08^{Bb}
	Cow	6.50 ± 0.04^{Aa}	6.43 ± 0.17^{Aa}	5.35 ± 0.17^{Ba}
	Goat	6.17 ± 0.04^{Ab}	5.85 ± 0.24^{ABab}	5.42 ± 0.14^{Ba}
	Buffalo	5.77 ± 0.02^{Ac}	5.43 ± 0.05^{Bb}	5.38 ± 0.12^{Ba}
	Sheep	5.90 ± 0.02^{Ac}	5.35 ± 0.17^{Bb}	5.74 ± 0.14^{ABa}

Table 4. Color parameters of cow kefir samples enriched with 1% colostrum of different origin 14 days of storage. The values are expressed as the means ± standard deviations (SDs). Different uppercase letters within the same row indicate significant differences over storage time ($p < 0.05$), and different lowercase letters within the same column indicate significant differences among kefir types ($p < 0.05$).

was observed during storage, indicating a trend toward improved gel stability. These observations are consistent with previous studies reporting that the incorporation of protein-rich dairy systems strengthens the gel network and reduces syneresis[44]. The enhanced water binding and reduced syneresis in colostrum-enriched kefirs may be attributed to their higher protein and mineral contents, which promote stronger protein–water interactions and a more stable kefir structure during storage. Buffalo and goat milk contain higher amounts of casein micelles compared to cow milk, as their protein matrix has smaller pores and greater density, leading to an increased water-holding capacity[34,45].

As shown in Table 4, the L* values of the kefir samples ranged from 61.38 to 75.34. Importantly, all samples exhibited a significant decrease in lightness throughout the storage period, reflecting a gradual darkening of the kefir during refrigeration. This reduction may result from pigment oxidation, protein aggregation, or changes in the light-scattering properties of casein micelles. Similar findings have been reported in fermented milk systems, where pigment oxidation or protein aggregation caused a decrease in brightness during storage[46]. Studies on fermented dairy products have also indicated that the decline in L* values over time is associated with changes in the product microstructure[47]. Moreover, factors such as milk type, the fat-to-protein ratio, and added components play crucial roles in maintaining color stability[48]. In this context, the lower color loss observed in kefir enriched with colostrum might be associated with the additional protein, fat, and mineral components contributed by colostrum, which could promote a more stable milk matrix structure. At the beginning and end

of storage, the highest lightness value (75.34 ± 0.07) was observed in kefir containing buffalo colostrum, whereas the lowest value (66.84) was obtained in kefir made with cow colostrum. The type of colostrum used in the formulation significantly affected the lightness (L*) of the kefir samples ($p < 0.05$). The control sample (without colostrum) exhibited a lower lightness value compared to kefir samples containing buffalo and goat colostrum ($p < 0.05$). These results indicate that the type of colostrum contributes positively to the lighter color of kefir. A similar trend was also reported by Setyawardani et al.[38], who observed that the inclusion of colostrum in kefir formulations significantly influenced the lightness parameter (L*), with samples containing higher proportions of milk or buffalo colostrum exhibiting lighter coloration. Consistent with these findings, the present study demonstrated that the type of colostrum plays a decisive role in determining kefir color characteristics, contributing to increased lightness compared with that of the control sample.

With respect to the a* values, all kefir samples presented slightly negative readings, shifting toward green hues. However, the values tended to become less negative during storage, suggesting a subtle transition toward redness. At the beginning of storage, kefir supplemented with buffalo colostrum exhibited a higher a* value, whereas by the end of storage, a* lower value was observed in the sample produced together with sheep colostrum. These variations might be related to microbiological, biochemical, and physicochemical changes occurring during processing and storage, which can influence pigment stability and color development in fermented dairy products[49]. As shown in Table 4, the use of colostrum powder in kefir production markedly increased the intensity of the yellow color at the beginning of storage, with the highest b* value (6.50 ± 0.04) observed in kefir containing cow colostrum. The b* values ranged between 4.96 and 6.50, reflecting the yellowish tone characteristic of fermented milk products. A gradual decline in b* values during storage was observed, which is consistent with previous findings by Zájác et al.[50] and Gul et al.[48], who attributed such decreases to pigment degradation and pH-dependent changes in the milk matrix. At the end of storage, kefirs produced with different types of colostrum powder showed similar yellow tones, while the control sample exhibited the lowest b* value. The variation in b* values is likely associated with differences in riboflavin and carotenoid contents among colostrum types, as well as the dilution effect of higher fat content in buffalo colostrum[51]. Overall, the color stability of kefir during storage is influenced by milk type, compositional factors (protein, fat, carotenoids), and biochemical changes associated with fermentation and storage time. The addition of colostrum may improve color stability by enhancing the buffering capacity and matrix integrity of the product.

Table 5 presents the microbiological counts of kefir samples enriched with different types of colostrum powder on days 1, 7, and 14 of storage. The microbial populations identified in the kefir samples varied depending on the type of colostrum and the duration of storage. Throughout the storage period, yeast, Lactobacilli, and Lactococci counts ranged between 3.26 and 5.07, 6.98 and 7.65, and 7.96 and 8.63 log CFU/mL, respectively. At the beginning of storage, the yeast counts ranged between 3.26 and 4.26 log CFU/mL, while at the end of the 14th day, they varied between 3.38 and 5.07 log CFU/mL. The highest yeast count (5.07 log CFU/mL) was detected in the sample containing buffalo colostrum on day 14, whereas the lowest value (3.38 log CFU/mL) was observed in the control kefir. In general, yeast growth in kefir during storage shows limited variation in the literature. Magalhães et al.[52] reported an increase in yeast counts during storage, which was attributed to the decrease in lactic acid bacteria, creating more favorable conditions for yeast growth. Similarly, Puerari et al.[4] reported a transient increase in yeast populations in the early days following fermentation, followed by a gradual decline with prolonged storage. Conversely, Leite et al.[53] reported that yeast counts remained stable at approximately 5–6 log CFU/mL throughout storage. These observations are generally consistent with recent studies exploring

Parameter	Sample	Storage (day)		
		1	7	14
Yeast	Control	3.28 ± 0.02^{Ab}	3.48 ± 0.02^{Ad}	3.38 ± 0.00^{Ad}
	Cow	3.46 ± 0.38^{Bb}	4.12 ± 0.06^{Aab}	3.47 ± 0.03^{Bc}
	Goat	3.26 ± 0.17^{Bb}	3.79 ± 0.01^{Ac}	3.61 ± 0.01^{ABc}
	Buffalo	4.02 ± 0.02^{Ba}	4.35 ± 0.05^{Ba}	5.07 ± 0.27^{Aa}
	Sheep	4.26 ± 0.04^{Aa}	4.38 ± 0.03^{Aa}	4.36 ± 0.00^{Ab}
Lactobacilli	Control	7.59 ± 0.02^{Aa}	7.46 ± 0.14^{Aa}	6.98 ± 0.02^{Bb}
	Cow	7.41 ± 0.03^{ABa}	7.61 ± 0.13^{Aa}	7.34 ± 0.06^{ABa}
	Goat	7.39 ± 0.11^{Aa}	7.25 ± 0.05^{Aa}	7.35 ± 0.50^{Aa}
	Buffalo	7.57 ± 0.01^{Aa}	7.61 ± 0.01^{Aa}	7.22 ± 0.08^{Ba}
	Sheep	7.40 ± 0.37^{Aa}	7.65 ± 0.35^{Aa}	7.37 ± 0.07^{Aa}
Lactococci	Control	8.28 ± 0.09^{Aa}	8.11 ± 0.20^{Ab}	8.30 ± 0.10^{Aab}
	Cow	8.18 ± 0.05^{Aa}	8.51 ± 0.16^{Aa}	8.54 ± 0.08^{Aa}
	Goat	8.31 ± 0.18^{Aa}	8.18 ± 0.10^{Aab}	8.63 ± 0.08^{Aa}
	Buffalo	8.39 ± 0.28^{Aa}	8.58 ± 0.04^{Aa}	8.30 ± 0.10^{Aab}
	Sheep	7.96 ± 0.12^{Ba}	8.46 ± 0.04^{Aa}	8.31 ± 0.07^{Aab}

Table 5. Microbial changes in cow kefir samples enriched with 1% colostrum of different origin during storage (log CFU/mL). The values are expressed as the means ± standard deviations (SDs). Different uppercase letters within the same row indicate significant differences over storage time ($p < 0.05$), and different lowercase letters within the same column indicate significant differences among kefir types ($p < 0.05$).

the production of fermented dairy products from colostrum, in which kefirs enriched with colostrum milk were reported to have Lactobacilli and yeast counts of approximately 6.92–7.48 log CFU/mL and 5.54–6.55 log CFU/mL, respectively[38]. In another study, kefir samples supplemented with colostrum exhibited Lactococci and Lactobacilli counts of 8.61 and 8.35 log CFU/g, respectively[55]. Similarly, kefir produced from buffalo colostrum showed LAB and yeast counts of 2.85×10^6 and 2.16×10^5 CFU/mL, respectively. These findings provide a comparative context, suggesting that the microbial counts observed in the present study are within the range reported for colostrum-enriched kefirs.

These findings suggests that the symbiotic microbial structure of kefir grains contributes to microbial stabilization and maintains balance during storage. Overall, previous studies on kefir produced from cow milk have shown that yeast counts generally remain stable or exhibit minor fluctuations during storage, although a decrease may occur under conditions of high acidity and low temperature. This highlights the importance of the symbiotic interaction between yeasts and bacteria in maintaining microbial stability in kefir[54]. Notably, kefir samples containing buffalo and sheep colostrum showed higher yeast counts, which may have exerted a limited inhibitory effect on Lactococci counts, while Lactobacilli counts remained relatively stable across all samples.

In this context, animal colostrum contains native bacterial populations mainly associated with the mammary environment, whereas yeasts are not considered dominant members of colostrum microbiota[26,27]. Therefore, changes in yeast counts observed during kefir storage are more likely related to kefir fermentation dynamics rather than to colostrum-derived microorganisms. The Lactobacilli counts ranged from 6.98 to 7.61 log CFU/mL. By the end of the storage period, kefir samples containing goat and sheep colostrum exhibited relatively stable Lactobacilli counts, whereas a decreasing trend was observed in the control, cow, and buffalo samples. However, the differences among the types were not statistically significant ($p > 0.05$). Throughout the entire storage period, Lactobacilli counts remained above 7.0 log CFU/mL in all the samples except the control. Colostrum has been reported to contain various bioactive components that may support the survival of lactic acid bacteria; therefore, the generally higher and more stable Lactobacilli counts observed in colostrum-supplemented kefir samples compared to the control may be associated with this effect[38,55]. Similarly, the Lactococci population remained high (7.96–8.63 log CFU/mL) throughout storage, with no pronounced decline in any sample. In colostrum-supplemented kefirs, Lactococci counts were slightly increased or maintained compared to the control, suggesting that the kefir matrix, together with the presence of colostrum, provided a favorable environment for their persistence. Overall, these results indicate that both Lactobacilli and Lactococci are well adapted to the kefir system and can maintain their viability during refrigerated storage.

It has been reported that colostrum is not a microbiologically inert material and harbors diverse microbial communities[26]. Therefore, the potential contribution of native colostrum microbiota to the microbial profiles observed in kefir cannot be completely excluded. However, in the present study, colostrum powders were incorporated at a relatively low level (1%, w/v), kefir was produced using pasteurized milk, and fermentation was carried out with a defined commercial starter culture. Under these conditions, microbial activity during fermentation and storage is considered to be largely driven by the starter microorganisms. This interpretation is further supported by the relatively stable and high counts of Lactobacilli (6.98–7.61 log CFU/mL) and Lactococci (7.96–8.63 log CFU/mL) observed across all samples. Accordingly, the microbial ecosystem of kefir appears to be primarily governed by starter culture activity, while the influence of native colostrum microbiota is likely limited. The lack of prior microbiological characterization of colostrum is acknowledged as a limitation of this study.

The relatively stable Lactobacilli and Lactococci counts observed in colostrum-enriched kefirs may be attributed not only to the increased availability of nutrients but also to the enhanced buffering capacity provided by colostrum. The protein and mineral content of colostrum may mitigate excessive pH reduction during storage, creating a more favorable environment for lactic acid bacteria survival. This buffering effect is particularly relevant in kefir, where microbial balance is strongly influenced by acidity. Additionally, colostrum contains bioactive compounds such as lactoferrin and immunoglobulins, which have been reported to exert selective antimicrobial effects, potentially limiting yeast overgrowth while supporting lactic acid bacteria viability. According to the data, Lactobacilli and Lactococci counts remained generally higher in colostrum-enriched kefirs compared to the control, without statistically significant differences between colostrum types ($p > 0.05$). However, kefir samples containing buffalo and sheep colostrum showed a significant increase in yeast counts, which may have exerted a limited inhibitory effect on Lactococci counts. Overall, these results indicate that fermentation in kefir is primarily dominated by the starter culture, and the addition of colostrum contributes positively to microbial stability. Future studies incorporating microbiological characterization of colostrum prior to fermentation and comprehensive profiling of fermented kefir would provide a clearer understanding of the respective contributions of starter and colostrum-derived microorganisms.

A total of 34 aroma compounds were identified in kefir samples enriched with goat, sheep, buffalo, and cow colostrum (Table 6). The distribution of these compounds varies significantly depending on the animal from which the milk was obtained. The richest profile was found in kefir enriched with sheep colostrum (34 compounds), followed by kefir enriched with cow colostrum (33 compounds), kefir enriched with goat colostrum (29 compounds), and kefir enriched with buffalo colostrum (24 compounds). When aroma compounds were categorized, the main aroma classes were found to be alcohols, ketones, acids, and aldehydes. More alcohol types were detected in sheep and cow kefir, while fewer alcohol types were detected in kefir enriched with goat and buffalo colostrum. Acids constituted the largest compound group in all samples, with acetic acid, butanoic acid, hexanoic acid, octanoic acid, nonanoic acid, and pentadecanoic acid being particularly prevalent. In terms of aldehydes, kefir enriched with sheep and cow colostrum showed greater diversity, containing eight different aldehydes. In contrast, only four aldehyde compounds were detected in kefir enriched with goat colostrum, and only two aldehyde compounds were detected in kefir enriched with buffalo colostrum. Similar aroma compounds have also been detected in different kefir samples in previous studies[10,52].

	Compounds	Control	Sheep	Cow	Goat	Buffalo
	Alcohols					
1158	1-Butanol	65.0 ± 2^{e}	134.2 ± 2^{b}	70.6 ± 3^{d}	207.4 ± 5^{a}	124.3 ± 9^{c}
1184	3-Penten-2-ol	1664.3 ± 14^{d}	1667.3 ± 15^{d}	1809.1 ± 21^{c}	2755.2 ± 18^{b}	8016.2 ± 43^{a}
1234	1-Pentanol	39.0 ± 3^{c}	192.7 ± 14^{a}	42.4 ± 2^{b}	17.1 ± 0.3^{d}	ND
1303	2-Hexanol	816.3 ± 9^{d}	785.4 ± 9^{e}	887.3 ± 12^{c}	1537.8 ± 19^{b}	3539.3 ± 15^{a}
1318	2-Methyl-2-buten-1-ol	182.8 ± 5^{d}	57.4 ± 5^{e}	198.7 ± 6^{c}	317.1 ± 13^{b}	541.1 ± 9^{a}
1355	1-Hexanol	50.1 ± 5^{b}	77.4 ± 2^{a}	54.5 ± 4^{b}	56.5 ± 5^{b}	53.2 ± 4^{b}
1370	4-Octanol	ND	98.6 ± 8^{a}	ND	22.2 ± 0.1^{b}	ND
1448	1-Heptanol	54.4 ± 3^{b}	2522 ± 18^{a}	59.1 ± 6^{b}	ND	ND
1540	2,3 Butanediol	77.2 ± 4^{d}	236.2 ± 8^{b}	83.9 ± 5^{c}	412.3 ± 32^{a}	84.5 ± 5^{c}
1578	1-Octanol	83.1 ± 7^{c}	589.2 ± 6^{a}	90.3 ± 8^{b}	38.4 ± 3^{d}	ND
2191	1-Tetradecanol	637.6 ± 5^{b}	142.1 ± 7^{c}	693 ± 6^{a}	145.4 ± 11^{c}	85.6 ± 4^{d}
	Acids					
1439	Acetic acid	2021.3 ± 16^{e}	2099.3 ± 11^{d}	2197.1 ± 18^{c}	9499.1 ± 50^{b}	$12{,}932.5 \pm 61^{a}$
1544	2-Methylpropanoic acid	36.1 ± 2^{c}	363.3 ± 13^{b}	39.2 ± 4^{c}	25.2 ± 4^{d}	466.6 ± 9^{a}
1620	Butanoic acid	418.8 ± 21^{e}	664.3 ± 14^{c}	455.2 ± 19^{d}	1285.2 ± 8^{b}	2150.3 ± 18^{a}
1672	3-Methylbutanoic acid	45.0 ± 2^{d}	455.3 ± 28^{a}	48.9 ± 4^{c}	36.7 ± 1^{e}	85.3 ± 5^{b}
1722	Pentanoic acid	189.0 ± 11^{c}	295.4 ± 28^{a}	205.4 ± 13^{b}	66.4 ± 0.8^{e}	161.5 ± 11^{d}
1839	Hexanoic acid	1083.3 ± 8^{e}	2474.6 ± 11^{b}	1177.5 ± 10^{d}	2364.4 ± 16^{c}	3596.4 ± 15^{a}
1951	Heptanoic acid	435.7 ± 12^{c}	1382.6 ± 18^{a}	473.6 ± 16^{b}	174.3 ± 12^{d}	129.4 ± 8^{e}
2060	Octanoic acid	1201.4 ± 10^{e}	5473.1 ± 2^{a}	1305.9 ± 11^{d}	2129.1 ± 14^{c}	2535.6 ± 11^{b}
2177	Nonanoic acid	7983.0 ± 14^{c}	9382.5 ± 23^{a}	8677.2 ± 17^{b}	3250.2 ± 13^{d}	1556.7 ± 11^{e}
2286	Decanoic acid	580.7 ± 13^{e}	920.6 ± 21^{c}	631.2 ± 17^{d}	1009.3 ± 17^{b}	1482.3 ± 18^{a}
2420	Benzoic acid	230.3 ± 15^{d}	500.9 ± 1^{c}	250.5 ± 20^{d}	588.4 ± 30^{b}	630.1 ± 49^{a}
2510	Dodecanoic acid	568.1 ± 5^{c}	625.3 ± 5^{b}	617.5 ± 7^{b}	522.2 ± 3^{d}	719.2 ± 6^{a}
2818	Pentadecanoic acid	$20{,}815.0 \pm 53^{d}$	$46{,}200.0 \pm 58^{a}$	$22{,}625.0 \pm 48^{c}$	$29{,}974.0 \pm 58^{b}$	$19{,}507.0 \pm 17^{e}$
	Aldehydes					
1113	Hexanal	432.9 ± 11^{c}	1304.4 ± 13^{a}	470.5 ± 12^{b}	119.3 ± 7^{e}	228 ± 15^{d}
1192	Heptanal	172.5 ± 5^{c}	239.2 ± 8^{a}	187.9 ± 7^{b}	121.4 ± 13^{d}	ND
1285	Octanal	140.2 ± 5^{c}	979.3 ± 12^{a}	152.4 ± 5^{b}	ND	ND
1390	Nonanal	79.9 ± 5^{b}	2425.3 ± 25^{a}	86.8 ± 4^{b}	ND	ND
1440	2-Octenal	25.7 ± 3^{b}	199.4 ± 1^{a}	27.9 ± 2^{b}	ND	ND
1492	Decanal	8.1 ± 1^{c}	416.5 ± 15^{a}	8.8 ± 0^{c}	29.3 ± 1^{b}	ND
1541	(E)-2-Nonenal	25.4 ± 3^{b}	934.3 ± 8^{a}	27.6 ± 2^{b}	ND	ND
1639	(E)-2-Decenal	2594.4 ± 21^{c}	8130.5 ± 28^{a}	2820.4 ± 24^{b}	1251.2 ± 11^{d}	883.4 ± 15^{e}
	Ketones					
1268	3-Hydroxy-2-butanone—(Acetoin)	$52{,}601.9 \pm 52^{d}$	$45{,}370.0 \pm 72^{e}$	$57{,}176.0 \pm 59^{c}$	$89{,}325.0 \pm 181^{b}$	$142{,}725.0 \pm 131^{a}$
1340	3-Hydroxy-2-pentanone	41.7 ± 2^{e}	115.5 ± 3^{c}	45.3 ± 0.8^{d}	149.3 ± 11^{b}	279.3 ± 21^{a}

Table 6. Aroma compounds of cow kefir samples enriched with 1% colostrum of different origin (µg/kg). The values are expressed as the means ± standard deviations (SDs) and different lowercase letters within the same row indicate significant differences among kefir types ($p < 0.05$). ND; Not detected

A total of 11 different alcohol compounds were identified in kefir enriched with cow colostrum. The samples containing the highest number of alcohol compounds were obtained from kefir enriched with sheep colostrum (11 compounds), while the samples containing the lowest number of alcohol compounds were obtained from kefir enriched with buffalo colostrum (7 compounds). The alcohol compound with the highest concentration was 3-penten-2-ol, followed by 2-hexanol. Both compounds were predominantly present in kefir enriched with buffalo colostrum. The overall distribution showed that 1-butanol was present in kefir produced from all milk types, while 1-pentanol was found in milk kefir samples enriched with cow, sheep, and goat colostrum, but not in samples enriched with buffalo colostrum. 1-Heptanol was present only in kefir from sheep and cows, and was not found in samples from other species. 2-methyl-2-buten-1-ol was detected in milk kefir enriched with the colostrum of cows, sheep and goats, but not in milk kefir enriched with buffalo colostrum. Previous studies, including those by Dan et al.[57], identified 1-hexanol and 1-heptanol as the primary alcohols in fermented milk using SPME–GC–MS, which is consistent with the findings of this study. Rutkowska et al.[58] also reported the use of 1-hexanol, 1-pentanol, and 2,3-butanediol in traditionally produced kefir. Ströher et al.[17] detected 2,3-butanediol at every storage stage as one of the main components of the alcohol group in kefir samples produced by traditional methods. Sánchez-Rodríguez et al.[59] reported that 1-hexanol was consistently detected among the volatile compounds in their analysis of goat milk kefir, and that its amount increased as fermentation

progressed. Conversely, Wang et al.[10] emphasized that low-level alcohol compounds (such as 1-butanol, 1-hexanol, and 1-heptanol) are important as they impart pleasant oily, fruity, and sweety aromas to kefir.

A total of 13 different acid compounds have been identified in kefir enriched with cow colostrum. These compounds are the most dominant in all kefir samples, and their amounts vary depending on the milk from which they are obtained. Similarly, Ströher et al.[17] emphasized that the volatile acid compounds found in kefir are products of carbohydrate and lipid metabolism and are present in high concentrations in kefir samples. They also noted that the concentrations of these acids varied significantly between different kefir samples. Pentadecanoic acid was identified as the most abundant compound in this group. Its concentration is much higher in enriched sheep with colostrum kefir than in other samples. Other notable compounds in the acid fraction include nonanoic acid, hexanoic acid, and acetic acid. Enriched buffalo with colostrum kefir contains particularly high concentrations of butanoic acid and octanoic acid, while heptanoic acid is found at the highest levels in enriched sheep with colostrum. Benzoic acid was found at low levels in all milk samples, with relatively high amounts detected in the enriched sheep and goat with colostrum kefirs. Walsh et al.[60] inoculated cow milk with kefir grains from various geographical locations and examined aroma development over a 24-h fermentation period. Using GC–MS to analyze volatile compounds, they identified substances such as acetic acid, hexanoic acid, octanoic acid, nonanoic acid, and decanoic acid, which exhibited significant changes during fermentation. Dertli and Çon[61] examined the microbial diversity of traditional kefir grains and their role in kefir aroma, and identified the acids present in kefir samples.

A total of eight different aldehyde compounds have been detected in kefir enriched with colostrum from various animals. Among these compounds, (E)-2-decenal was found at the highest levels, with particularly high concentrations recorded in kefir enriched with sheep colostrum. Nonanal and hexanal are also important aldehyde compounds, and again the highest levels were recorded in kefir enriched with sheep colostrum. Heptanal was detected in all types except kefir enriched with buffalo colostrum. Octanal, 2-octanal, decanal, and (E)-2-nonenal were found in kefir enriched with cow and sheep colostrum, but not in kefir enriched with goat or buffalo colostrum. Overall, the richest aldehyde profile was observed in kefir enriched with sheep colostrum, while only a limited number of compounds were detected at low concentrations in other types. Vagenas and Roussis[62] detected heptanal and nonanal in cow, sheep, and goat milk products. Pluta-Kubica et al.[63] examined the volatile profile of fresh and stored cheeses made from sheep's milk, detecting the (E)-2-decenal compound independently of storage time. Sanchez-Rodriguez et al.[59] reported the presence of nonanal and hexanal in goat milk kefir. Meral-Aktaş et al.[64] found that the presence of hexanal, nonanal and decanal in kefir produced from cow, buffalo, sheep and goat milk was due to the oxidation of milk fat or the decomposition of amino acids.

This study identified two different ketone compounds in kefir. The most dominant of these was 3-hydroxy-2-butanone (acetoin), which was found at the highest levels in kefir enriched with buffalo colostrum. This compound was found in high concentrations in all types of kefir. However, significantly higher values were recorded in milk kefir enriched with buffalo and goat colostrum compared to milk kefir enriched with cow colostrum. The second compound, 3-hydroxy-2-pentanone, was detected at relatively low levels; the highest value was measured in kefir enriched with buffalo colostrum, while the lowest value was measured in kefir enriched with cow colostrum. Acetoin is one of the most commonly reported ketones in fermented milk products. Walsh et al.[60] demonstrated that acetoin is a key aroma component in kefir samples. Furthermore, McSweeney and Sousa[56] emphasized in their review of cheese ripening that acetoin is one of the main ketones involved in the aroma development of dairy products. Meral-Aktaş et al.[64] reported high concentrations of acetoin in kefir made from the milk of cows, buffalos, sheep, and goats. Overall, the acetoin and 3-hydroxy-2-pentanone detected in colostrum kefir correspond to the main ketones identified in kefir and other dairy products in the literature. These ketone compounds contribute significantly to the aromatic profile of dairy products due to their low detection thresholds, generally imparting positive sensory characteristics associated with cheese aroma[17].

PCA

In this study, covariance-based principal component analysis (PCA) and a heatmap graph created using OriginPro 2024 software to examine how kefir samples produced from colostrum belonging to different animal species differ in their volatile compound profiles (Fig. 1). The PCA results showed that PC1, which explained 94.61% of the total variance, determined the fundamental aromatic differences between the samples, while the secondary variations were reflected by PC2 (5.12%) and PC3 (0.24%). The three-dimensional PCA score graph showed that control group and cow kefir were clustered closely together in the negative direction of the PC1 axis and clearly separated from all the other samples. Since both the control and the cow colostrum-enriched milk kefir are produced from cow's milk, they naturally have very similar aromatic compositions. In contrast, sheep colostrum-enriched milk kefir was positioned in the positive directions of both PC1 and PC2, indicating a richer and more intense volatile aroma profile. Goat colostrum-enriched milk kefir showed a positive deviation on the PC2 and PC3 axes, indicating a distinctive aromatic profile, and buffalo kefir was centred around the axes, suggesting a balanced distribution of volatile compounds. The dataset was evaluated on a $\log_{10}$ scale in the heatmap analysis. PCA analysis supported the findings; specifically, buffalo colostrum-enriched milk kefir was characterized by high concentrations of certain medium- and long-chain acids and ketones, while control group and cow colostrum-enriched milk kefir had the simplest aromatic profile with low levels of most compounds. These results clearly demonstrate through PCA and heatmap analyses that the aromatic compound compositions of kefirs differ significantly depending on the type of colostrum used.

Conclusion

In this study, the use of lyophilized colostrum powders (1%) obtained from different animal species for kefir production positively influenced the overall quality of the product. The addition of colostrum increased the total solids content, reduced the degree of serum separation, and improved water-holding capacity. Kefirs enriched

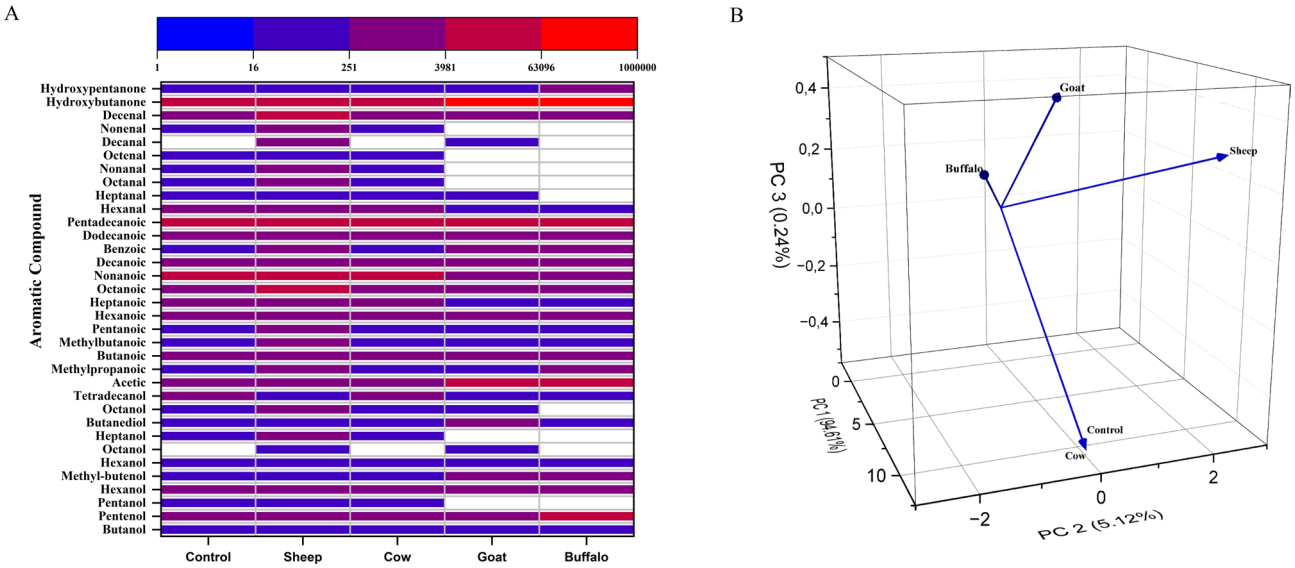

Fig. 1. **A**: Heatmap graph, **B**: The 3D PCA graphs.

with buffalo and goat colostrum demonstrated enhanced water retention properties. In terms of color, colostrum supplementation limited the loss of lightness and helped preserve visual quality during storage. Changes in the a* and b* values were associated with pigment–matrix interactions during storage. Microbiological analyses revealed that the addition of colosrum supported the viability of lactic acid bacteria, while goat and sheep colostrum in particular contributed to maintaining Lactobacilli stability. A total of 34 aroma compounds were identified in kefir supplemented with colostrum from goats, sheep, buffalo and cows, ranked as follows: 34 aroma compounds from sheep, 33 from cows, 29 from goats and 24 from buffalo. Acids formed the largest group of compounds in all the samples, with acetic acid, butanoic acid, hexanoic acid, octanoic acid, nonanoic acid, and pentadecanoic acid being particularly prevalent. Kefir supplemented with sheep and cow colostrum exhibited greater diversity, containing eight different aldehydes. This was followed by milk kefir enriched with goat colostrum containing four aldehydes and milk kefir enriched with buffalo colostrum containing two aldehydes. In general, the addition of colostrum powders as an ingredient in kefir production has the potential to develop new dairy products.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

Informed consent
The general objectives of the study were explained to the farmers/operations and colostrum samples were collected from those who signed a consent form approving the use of the obtained data for research purposes.

Human or animals rights
We confirm that permission to collect the colostrum was obtained from the owners of the animals, i.e. the farmers/operations.

Additional information

Correspondence and requests for materials should be addressed to A.C.

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