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Proximate and fatty acid analysis of goat and goat-cow mixed milk cheeses

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ABSTRACT

Goat milk is a valuable resource for food production thanks to its physical, chemical, and biological properties, easy digestibility, and lower allergenicity. The dairy product market in Kazakhstan is growing, leading to advancements in the industrial processing of goat milk. Consequently, this study aimed to analyse the proximate composition of raw goat milk, produce cheeses from goat milk alone and in combination with cow milk and examine the fatty acid profiles of the resulting cheeses. The findings indicated that goat milk contained a higher percentage of protein, while a 1:1 mixture of goat and cow milk exhibited increased levels of lactose and fat (p <0.001). A soft cheese prepared from goat milk exhibited faster coagulation, higher cheese yield and fat in dry matter. Furthermore, the saturated fatty acid (SFA) content was greater in cheeses made from the goat-cow milk blend compared to those made solely from goat milk (p <0.05). Notably, an increased presence of oleic acid and polyunsaturated fatty acids (PUFAs), such as linoleic, linolenic, and palmitoleic acids, was only detected in 100% goat milk cheeses. Overall, the soft cheese produced from goat milk showed improved nutritional qualities, particularly regarding fatty acid content. However, additional research is needed to assess sensory attributes and consumer acceptance.

Keywords: goat milk, cow milk, cheese yield, nutritional analysis

INTRODUCTION

At present, the domestic market of dairy products is developing and industrial processing of goat milk is evolving. Goat breeds such as Zaanen goat, Alpine goat, and Nubian goat are the most used dairy breeds in Kazakhstan. Goat's milk is of interest because of its physicochemical and biological properties, as it is easier to digest and is considered less allergenic **[1]**. The fat content of goat's and cow's milk is almost at the same level at around 3.5-4% **[2]**. Previous research has shown that goat and camel milk is better digested than buffalo and cow milk because of the small size of the fat globules **[3]**. The fat content of goat's milk has higher short- and medium-chain triglycerides than cow's milk fat **[4]**. Creating biotechnologies of nutritious food products is a priority and the most important area of development of the dairy industry. Currently, the range of goat milk products is limited **[5]**. The peculiarity of goat's milk cheese production is due to its lower ability to be coagulated by enzymes, which, to some extent, can be explained by the fractional composition of the protein and low titratability **[6]**. Therefore, cow milk or the introduction of increased doses of bacterial starter and calcium chloride are often used to ripen goat milk when processing goat milk into cheese **[7]**. Using goat's milk in producing soft cheeses, possessing hypoallergenic and special biological properties, is of certain scientific and practical interest. López-Villafaña et al. analysed the antioxidant activity, which was stronger in Mexican Panela cheeses manufactured from goat milk **[8]**. In addition, the Akavi and Halloumi cheeses from goat milk have been studied previously **[9]**.

Recent efforts have focused on creating goat milk cheese with altered texture properties. For example, fresh goat cheese was developed by incorporating polysaccharides to meet consumer desires and production requirements **[10]**. The strain of *Lactococcus lactis* has been utilised to enhance the quality and safety of goat fresh cheese while maintaining natural farming methods **[11]**. Thus, the research addressed growing consumer interest in the health benefits and nutritional differences between goat and goat-cow milk cheese, particularly regarding their fatty acid profiles, which can influence dietary choices and health outcomes. The paper presents the possibility of using goat's milk as the main component in cheesemaking in a ratio to cow's milk. This work aimed to develop soft brine cheeses from 100% goat and mixed (1:1) goat and cow milk and to study their approximate composition. The practical impact of this study lies in providing valuable insights for consumers, nutritionists, and food producers, enabling informed decisions that cater to health preferences and dietary needs.

Scientific Hypothesis

We expect significant differences in proximal composition and fatty acid composition between goat and cow milk blends and soft cheeses prepared on their basis. We hypothesise that goat milk will exhibit a higher concentration of PUFAs than cow milk blends, which are expected to contain more SFAs.

MATERIAL AND METHODOLOGY

Samples

The studies were conducted in the laboratory of the “Aq Altyn” cow milk processing dairy enterprise. Goat milk from Alpine and Zaanen breeds in the Zhambyl region of Kazakhstan was used as raw materials to make soft cheeses.

Chemicals

Isoamyl alcohol (purity ≥99.9%), distilled water, and sodium hydroxide (purity ≥99%) were retrieved from LPP Labhimprom (Almaty, Kazakhstan). 37-component FAME Mix standard from Supelco, Merck (Darmstadt, Germany), nitrogen (purity ≥99%) (Hauppauge, NY, USA), sodium methylate powder (Sigma-Aldrich, St. Louis, MO, USA), absolute methanol (Sigma Aldrich, St. Louis, MO, USA), MikroMilk YO 100 cultures, MicroMilk MF2.250 enzyme (microMilk S.r.l., Italy), $CaCl_2$ (Russia). All chemicals were of analytical grade quality.

Instruments

Milk Analyzer Ekomilk BOND (Bultech2000, Bulgaria), Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan), laboratory liquid thermometer (Termex, Russia), flasks (K-1-250-29/32) and glasses (V-1-150 TS) (Kazlabpribor, Kazakhstan), class 4 laboratory scales (Mertech, Kazakhstan), water bath (Stegler, US).

Laboratory Methods

Samples of milk and finished products were analysed using the standard methods. The technical conditions for raw goat and cow milk were analysed according to the **[12]** GOST 32940-2014 and GOST 31449-2013 **[13]**. Fat content, acidity, and milk density were carried out based on the **[14]** GOST 5867-90, **[15]** GOST 3624-92 and **[16]** ST RK 1483-2005, respectively. Technical conditions for brine cheeses were performed according to the **[17]** GOST-33959-2016. The determination of fatty acid content was analysed by gas chromatography **[18]**. The following formula determined the cheese yield:

$$Vpr = (Mg.pr)/Ms \times 100\%,$$

Where:
Vpr – product yield; %; Mg.pr – mass of the finished product; g; Ms is mass of initial raw material, g.

Description of the Experiment

Sample preparation: The goat milk was cooled to 4 °C and transported in a thermal container capable of maintaining product temperature for 48 hours.

Number of samples analyzed: 8

Number of experiment replication: 3

Design of the experiment:

Soft goat cheese manufacture: All cheese samples were obtained according to the traditional technology of brine cheese production. Milk filtration was carried out to purify from mechanical impurities. After that, milk samples were sent for separation at 40 °C and subjected to normalization by mass fraction of fat. A milk ratio of 1:1 was produced for mixed milk samples. Next, the milk samples were homogenized to obtain a homogeneous mixture at 15 MPa, pasteurized at 72-74 °C with a holding time of 20-25 sec, and cooled to leavening temperature

34 °C. Furthermore, to give taste characteristics lyophilized starter cultures of direct application of MikroMilk YO 100 (pure cultures of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* in the ratio of 1:1) in the amount of 1-3% of the weight of milk was introduced. Then, calcium chloride (CaCl2) was used to restore calcium balance after pasteurization and improve rennet coagulation. MicroMilk MF2.250 was used to coagulate the mixture, and a standardised powder of 100% chymosin was obtained by fermentation of Rhizomycor Miehei using a VNIIMS mug. Milk coagulation was carried out for 30-40 minutes until a dense clot was obtained; staging was done by cutting the clot into cubes of equal size. We carried out moulding and self-pressing, salting in brine for a day at a salt concentration of 20%, incubation in brine at a concentration of 18% for 4 days, realisation in brine (in a barrel or in any other container) or packing in vacuum bags. The shelf life of the finished product in brine is 75 days, and in vacuum packaging, it is 50 days at a temperature of no more than 6 °C.

Determination of fatty acid content by gas chromatography: The cheese samples were securely sealed and kept in a refrigerator at a temperature of +2 degrees for 8 hours during its transfer from the workshop to the laboratory. Before being shipped, the cheese samples were soaked in brine for 24 hours, containing a table salt concentration of 19-21%. Around 100-150 g of shredded cheese was combined with a solution of n-hexane and sodium methylate in methanol. After allowing the methylation reaction mixture to settle for 5 minutes, it was centrifuged at 3000 rpm for an additional 5 minutes. Following this, 1 mL of the resulting supernatant was transferred to a vial for gas chromatography analysis. The measurements were then conducted using chromatography. The fatty acid methyl esters (FAMEs) were analysed using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) featuring a flame ionisation detector **[18]**. A high-polarity CP-Sil 2560 fused silica column (100 m × 0.250 mm × 0.20 µm; Agilent Technologies, Santa Clara, CA, USA) was employed for the chromatographic analysis of FAMEs. The gas chromatography experiment was conducted with the following settings: an injector temperature of 250 °C and a detector temperature of 260 °C. The analysis was performed in split mode with a ratio of 1:40, and the total flow rate was set at 95.5 mL/min. The temperature program for the column began at 100 °C, maintained for 5 minutes. The temperature was then increased at a rate of 4 °C/min until it reached 210 °C, where it was held for 8 minutes. Subsequently, the temperature was raised by 10 °C/min to reach 240 °C, which was then held for an additional 16.5 minutes. An injection volume of 1.0 µL was used, resulting in a total analysis duration of 60 minutes. The carrier gas used was nitrogen generated by a Parker Domnick Hunter G1110E nitrogen generator (Hauppauge, NY, USA). The hydrogen flow rate was set at 30 mL/min, while the air flow rate was 300 mL/min, with the remaining flow also at 30 mL/min. To quantify the FAMEs, the 37-component FAME Mix standard from Supelco, Merck (Darmstadt, Germany) was utilised. Each component's concentration was calculated by normalising its peak area against the total peak area, thus expressing the relative abundance of each compound **[19]**. The analysis of fatty acids (FAs) using the gas chromatograph equipped with a flame ionisation detector (GC/FID) method was validated following International Conference on Harmonisation (ICH) guidelines **[20]**. Each FAME component was identified based on the retention times and chromatograms obtained from the standard mix. The method's precision was evaluated through repeatability, which involved performing the procedure five times with the standard mix solution. The precision of the chromatographic system was confirmed by calculating the %RSD for retention times and peak areas, with five injections made across three different days.

Statistical Analysis

The fatty acid composition was analysed using Hierarchical Cluster Analysis (HCA), employing Euclidean distances and Ward's method. Since our study compares the mean between two independent groups, we used independent samples t-test in the SPSS v25.0 software (IBM Corporation, New York, USA). Outcomes with p <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Organoleptic and physicochemical properties of tested milk samples

Goat's milk has been demonstrated as an alternative for people sensitive or allergic to cow milk **[21]**. It can be seen from the data presented in Table 1 that the organoleptic data of the mixture of cow and goat milk (GCM) was similar to the goat milk (GM) samples. The fat mass fraction and lactose content of the GCM were 5.2% and 4.6%, which are significantly higher than that of GM (p <0.05). Compared to pure goat milk, the higher fat mass fraction and lactose content in goat and milk blends can be attributed to the composition and properties of the additional milk used, often cow's milk, which typically has a higher lactose concentration and fat content than goat milk. Previous studies detected higher lactose content in cow or human milk samples than in goat milk **[22]**, **[23]**. According to the meta-analysis, the average goat milk lactose was 4.44% **[24]**. Importantly, the mass fraction of protein was higher (3.21%) in GM than in GCM (p <0.001). Goat milk has a higher protein content than cow milk due to its unique casein profile, which contributes to better digestibility and a higher concentration of essential amino acids. Differently, greater protein content was observed in sheep milk cheese than in goat milk cheese **[25]**. In addition, GM showed enhanced TA (p <0.001), while no significant changes were found for pH. In general, titratable acidity indicates the freshness of the milk **[26]**.

Table 1 Organoleptic and physicochemical properties of tested milk samples.

Indicators	100% GM	1:1 GCM
Color	Yellowish tinge	Pale yellow
Taste and odor	Clean, without any foreign odors or flavors not characteristic of fresh goat's milk	Clear, without any odors or tastes not typical of fresh goat's milk
Consistency and appearance	Homogeneous liquid without sediment and protein flakes	Homogeneous liquid without sediment and protein flakes
Mass fraction of fat, %	^{b}4.91 ±0.02	^{a}5.2 ±0.12
Mass fraction of protein, %	^{A}3.21 ±0.01	^{B}2.98 ±0.008
Density, kg/m3	1.29 ±0.008	1.29 ±0.001
Titratable acidity (TA), °T	^{A}18 ±0.12	^{B}16.91 ±0.04
Lactose, %	^{B}4.06 ± 0.01	^{A}4.6 ±0.12
Acidity, pH	6.61 ±0.004	6.5 ±0.15
Freezing point, °C	^{A}56.2 ±0.07	^{B}54.08 ±0.06
Temperature, °C	^{A}24.2 ±0.2	^{B}22.95 ±0.02

Note: different letters indicate significance, a, b (p <0.05); A, B (p <0.001).

Physicochemical properties of tested brine cheese samples

The technological properties of the cheese samples are given in Table 2 and Figure 1 depicts the ready cheeses. It should be noted that coagulation in GMB was faster than in GCMB (Figure 2) (p <0.001). These data are important for cheese production because if the coagulation time is increased more than necessary, it will significantly affect the consistency of the cheese. This might be due to the higher concentration of certain coagulation factors, such as specific casein fractions and a more favourable micelle structure in goat milk, which promote more efficient aggregation and formation of curds during the cheese-making process **[27]**. In contrast, according to a previous study, buffalo and sheep milk demonstrate superior cheese-making qualities, including coagulation and curd firming properties, nutrient retention in the curd, and overall cheese yield, in comparison to bovine and caprine milk **[28]**. However, both breed and nutrition are critical in determining the coagulation properties of milk and the overall yield and quality of cheese produced. For instance, Italian local goats demonstrated better coagulation characteristics than Saanen goats **[29]**. Regarding cheese yield, cheese made from pure goat milk resulted in significantly higher cheese yield (p <0.001) (Figure 3). Meanwhile, high-temperature treatments of goat milk were observed to have a notable impact on the fundamental composition and protein composition of goat cheese whey **[30]**. Elgaml et al. reported that cheese prepared from 100% goat milk yielded higher Halloumi cheese yields than cow and mixed cheese samples **[31]**. Conversely, Algerian Bouhezza soft cheese yield was highest when made from sheep's milk, followed by goat's milk and cow's milk. This was notable despite the initial volume of milk used in the cheese-making process being nearly identical, suggesting that the processing methods significantly impact the final yield. **[32]**. The Nubian breed has been shown to produce more cheese than the Alpine breed, indicating that breed and other factors can influence the quality and quantity of dairy products **[33]**. Moreover, the colour of the cheese samples varied from white to yellowish, which indicated a difference in the mass fraction of fat in the samples (Table 2).

In our study, GMB showed higher fat content (45.13%) than GCMB (43.05%), which may be affected by cheese-making. In contrast, higher fat content was revealed in traditionally smoked cheeses made from cow milk than those made from sheep and goat milk **[34]**. Significantly greater moisture (53.06%) was found in GCMB samples (p <0.001). This result agrees with Gebreyowhans et al., who also revealed reduced moisture content in pure goat milk Camembert cheese than cheese made from cow milk **[35]**. Besides, our study did not detect differences in salt percentage and pH among samples (p >0.05).

Figure 1 Finished brine cheeses: top left 50:50% goat and cow's milk cheese and right 100% goat's milk cheese; bottom left 100% goat's milk cheese and right 50:50% goat and cow's milk cheese.

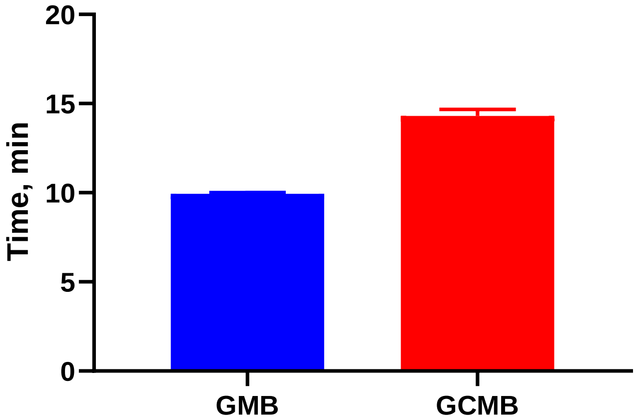


Figure 2 Beginning of coagulation time. GMB: based on goat milk, GCMB: based on 1:1 ratio of cow and goat milk.

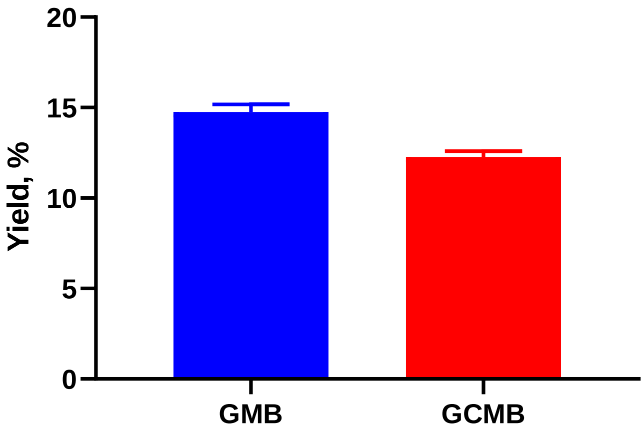


Figure 3 Cheese yield**.** GMB: based on goat milk; based on the ratio (1:1) of cow and goat milk.

Table 2 Technological properties of tested cheese samples.

Parameters	100% GMB	1:1 GCMB
Color	Yellowish	Faint yellow
Taste and odor	Pure, characteristic of goat's milk, creamy	Pure, characteristic of goat's milk, moderately acidic
Consistency	Homogeneous, slightly tender	moderately dense
Appearance	The outer layer is compacted, and the surface of the cheese is flat and slightly rough.	The outer layer is compacted, and the surface of the cheese is flat and slightly rough.
Drawing	A small number of irregularly shaped eyes throughout the mass	A small number of irregularly shaped eyes throughout the mass
Mass fraction of fat in dry matter, %	^{A}45.13 ±0.1	^{B}43.05 ±0.08
Mass fraction of moisture, %	^{B}49.98 ±0.05	^{A}53.06 ±0.06
Mass fraction of salt, %	1.51 ±0.01	1.50 ±0.007
Active acidity, pH	5.37 ±0.06	5.22 ±0.05

Note: different letters indicate significance, A, B (p <0.001).

Fatty acid profile of produced cheeses

Fatty acid composition is one of the most important indicators of the nutritional quality of goat milk and milk products. Table 3 and Figure 4 presents the data on cheeses' fatty acid composition. The amount of saturated fatty acids (SFAs) was higher in GCMB cheese than in pure goat milk cheese (p <0.05). In a previous study, the content of $C_{16:0}$ and $C_{18:0}$ was about 33.60-31.68% and 10.25-8.82% in goat cheese samples, respectively **[36]**. In addition, less $C_{18:0}$ was detected in hard goat's milk cheeses than in hard cheeses made from cow's or sheep's milk **[37]**. These results were also confirmed in Egyptian fresh soft cheese samples by Ibrahim et al. **[38]**. Therefore, the increase in saturated fatty acids is likely due to the mixing of cow milk with goat milk. Szterk et al. found that the levels of SFAs in the analysed commercial goat cheeses are relatively low. The authors also suggested that the type of cheese significantly influences the fatty acid composition, with soft cheeses being more likely to contain essential fatty acids **[39]**. In addition, it is suggested that palmitic acid should be consumed in a certain ratio with PUFAs to prevent the acceleration of deleterious effects **[40]**.

Regarding monounsaturated fatty acids (MUFAs), significantly more oleic acid ($C_{18:1n9c}$) was detected in goat milk cheese compared to mixed milk cheese (p <0.05). $C_{18:1n9}$ contents were higher in goat milk than human milk **[41]**. In contrast, the levels of MUFAs, particularly oleic acid, in goat and sheep cheese samples were similar, whereas cow cheese samples showed significantly higher amounts **[42]**. It is well-documented that oleic acid lowers lipid levels in the blood and has a positive effect that positively affects the cardiovascular system **[43]**.

Importantly, $C_{18:3n3c}$, $C_{16:1}$, $C_{18:2n6c}$ fatty acids were identified only in 100% goat milk sample cheeses. Commercial goat cheeses had the highest n-3 PUFA content, according to a study by Paszczyk and Łuczyńska **[37]**. However, it has been shown that sheep cheese exhibited a higher concentration of polyunsaturated fatty acids (PUFAs), including n-3 PUFAs, compared to cow or goat cheese **[44]**. PUFAs are involved in the structure and functioning of cell membranes **[45]**. Linoleic acid is an omega-6 fatty acid part of membrane phospholipids and serves as a structural component of membrane fluid to maintain the epidermal transdermal water barrier **[46]**.

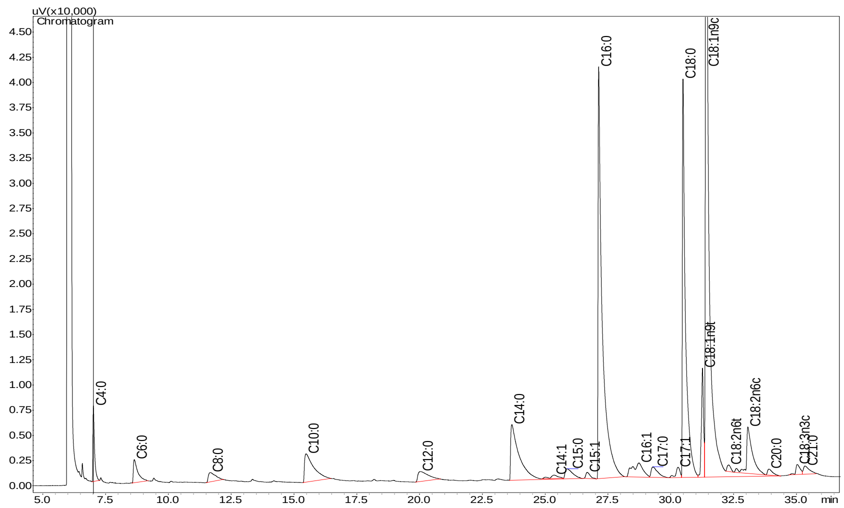
Furthermore, increased levels of linoleic acid were found in cheese-containing cultures in goat cheese **[47]**. It is also reported that incorporating extruded linseed into goats' diets enhanced the fatty acid composition of Padraccio cheese **[48]**. Thus, the fatty acid profile of goat cheeses can be significantly influenced by the goats' breed and diet, particularly the types of forage and supplements they consume. For instance, grazing led to notably increased levels of n-3 fatty acids in goat milk and cheese samples and a reduced n-6/n-3 ratio **[49]**. Additionally, factors such as the stage of lactation and the processing methods employed during cheese production can also alter the composition and ratios of fatty acids in the final product. Currò et al. reported that SFAs were abundant in early lactation, while unsaturated fatty acids were abundant in late lactation. Also, local goat breeds produced milk with a lower concentration of SAFs than the cosmopolitan breeds **[50]**.

Table 3 The fatty acid composition of cheese.

Fatty acid composition of fat phase, µg/mL	100% GMB	1:1 GCMB (50G:50C)
Saturated fatty acids		
$C_{16:0}$ palmitic acid	^{B}21.86 ±0.21	^{A}32.51 ±0.19
$C_{18:0}$ stearic acid	^{B}15.44 ±0.02	^{A}20.66 ±0.19
Monounsaturated fatty acids		
$C_{18:1n9c}$ oleic acid	^{A}35.29 ±0.05	^{B}31.87 ±0.04
$C_{21:0}$ henicosanoic acid	0.64 ±0	ND
Denatured fatty acids		
$C_{18:2n6t}$ linolelaidic acid	0.32 ±0.006	ND
Polyunsaturated fatty acids		
$C_{18:2n6c}$ linoleic acid	3.47 ±0.03	ND
$C_{18:3n3c}$ linolenic acid	0.56 ±0.008	ND
$C_{16:1}$ palmitoleic acid	1.9 ±0.02	ND

Note: ND – not detected.

A)



B)

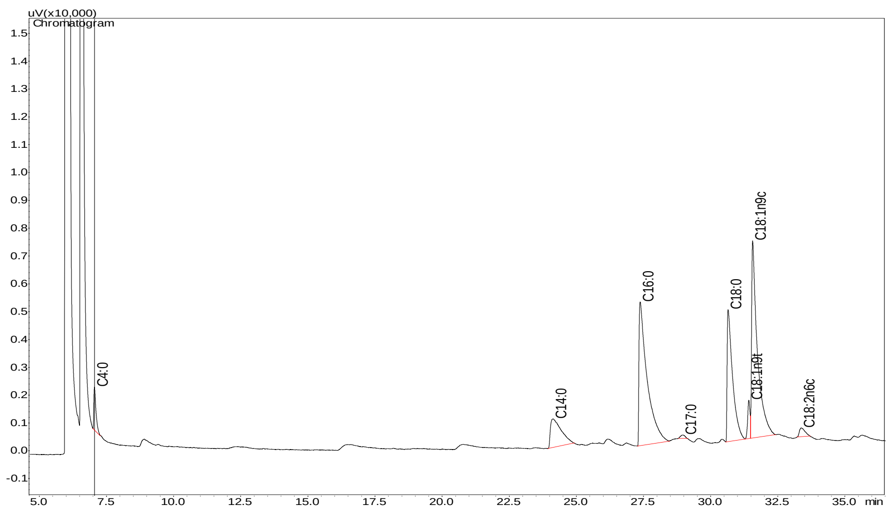


Figure 4 Chromatogram of cheese samples: A) 100% goat milk cheese; B) 1:1 goat and cow milk blend cheese.

CONCLUSION

The present results showed that goat milk samples exhibited a higher percentage of protein, while goat and cow milk blends showed greater lactose and fat content. Soft cheese prepared from goat milk exhibited faster coagulation, higher cheese yield and fat content in dry matter. The content of SFAs was higher in goat and cow milk cheeses than in those made from pure goat milk cheeses. In addition, increased content of oleic acid as well as PUFAs such as linoleic, linolenic and palmitoleic acids were found only in 100% goat milk cheeses. Soft cheese made from goat milk exhibited improved nutritional characteristics, especially concerning fatty acids. However, further research is required on sensory analyses and consumer acceptability.

The study revealed that goat milk-based cheeses offer significant nutritional benefits. The goat milk samples contained a higher protein content (3.21%) compared to the goat-cow milk mixture (2.98%), while the goat-cow milk blend had a higher fat content (5.2%) and lactose content (4.6%) compared to goat milk alone (4.91% fat, 4.06% lactose). The cheese yield was notably higher for goat milk cheeses ($p < 0.001$), and these cheeses also had a greater fat content in dry matter (45.13%) compared to cheeses made from a goat-cow milk mixture (43.05%).

The fatty acid analysis showed that saturated fatty acids (SFAs) were significantly higher in the goat-cow milk cheeses, with palmitic acid (C16:0) reaching 32.51 µg/mL and stearic acid (C18:0) at 20.66 µg/mL, compared to 21.86 µg/mL and 15.44 µg/mL, respectively, in pure goat milk cheeses. Meanwhile, pure goat milk cheeses contained higher levels of oleic acid (C18:1n9c, 35.29 µg/mL). They were the only ones to exhibit polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2n6c, 3.47 µg/mL), linolenic acid (C18:3n3c, 0.56 µg/mL), and palmitoleic acid (C16:1, 1.9 µg/mL).

These findings highlight the superior nutritional profile of 100% goat milk cheeses, particularly regarding their higher PUFA content, contributing to improved health benefits. Further research is necessary to explore sensory qualities and consumer preferences.

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