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Amino Acid and Fatty Acid Composition of Soft Cheeses

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Abstract

The purpose of this study was to determine the nutritional quality of various soft cheeses based on protein-building amino acid, free amino acid, and fatty acid composition. Buffalo, cow, and goat soft cheeses produced with traditional self-fermentation method from unpasteurized milk were investigated. Significant differences were observed in the amino acid composition of the three cheese types, with goat cheese having the highest amino acid content, followed by cow cheese, and buffalo cheese. The main amino acids found in soft cheeses are glutamic acid, proline, leucine, lysine, and aspartic acid. Goat and cow cheeses showed similar amino acid compositions. Buffalo cheese contained significantly higher amounts of methionine and a lower amount of histidine. Essential amino acids accounted for approximately 40% of the total amino acid content, indicating the high nutritional value of these cheeses. The free amino acid composition varied among cheeses, with gamma-aminobutyric acid, glutamic acid, lysine, proline, and aspartic acid being the main free amino acids. The fatty acid composition also differed, with goat cheese having higher proportions of short-chain fatty acids (caprylic acid, myristic acid), and saturated fatty acid (palmitic acid). Buffalo cheese had higher levels of saturated stearic acid and polyunsaturated linoleic acids, whereas cow cheese had the highest level of monounsaturated oleic acid. All investigated types of soft cheeses have nutritionally valuable compositions in terms of protein-building amino acids, free amino acids, and fatty acids.

Keywords: *Amino Acid; Fatty Acid; Cheese; Nutrition Value; Quality*

Abbreviations

BC: Buffalo Cheese; CC: Cow Cheese; FAA: Free Amino Acids; FAME: Fatty Acid Methyl Esters; FAO: Food and Agriculture Organization; FDM: Fat in Dry Matter; GC: Goat Cheese; MANOVA: Multivariate Analysis of Variance; MFFB: Moisture on a Fat-Free Basis; MUFA: Monounsaturated Fatty Acids; PCA: Principal Component Analysis; PUFA: Polyunsaturated Fatty Acids; SFA: Saturated Fatty Acids; WHO: World Health Organization

Introduction

There are thousands of types of cheese, classified according to different characteristics such as country or region of origin, type of milk, texture, maturation time, and fat content. Cheese can be produced from the milk of different animals (cow, buffalo, goat), which has an impact on the cheese's sensory characteristics and nutritional properties. Buffalo milk is richer in fat, lactose, protein vitamins, and minerals compared to cow milk [1], while goat milk is characterized for high levels of vitamin A, thiamine, and niacin [2]. Cow milk is widely recognized for its balanced composition and versatility in cheese production, making it a popular choice among cheesemakers worldwide.

Based on the texture of cheese, there are four main groups: extra hard, hard, semi-hard, and soft cheeses. Extra hard cheeses are characterized by low moisture (< 51% MFFB: moisture on a fat-free basis) and high fat (> 60% FDM: fat in dry matter) content. Hard cheeses have a moisture of 49 - 56% MFFB and the fat is 40 - 60% FDM, semi hard cheeses have a higher moisture content than hard cheese (54 - 69% MFFB) and lower in fat content (24 - 45% FDM). While soft cheese has a soft texture, which becomes viscous and creamy with ripening, it has high moisture > 67% MFFB, and the fat content accounts for 12 - 25% FDM [3].

Protein is one of the main components that affects the structure and nutritional value of cheese. The main protein-building amino acids detected in cheese are glutamic acid, proline, leucine, lysine and valine. Essential amino acids that are highly important from nutritional point of view account for 40% of total amino acid composition of cheese [4]. Amino acid composition of cheeses usually merits the recommendation of daily intake for adults [5]. Goat cheese has highest amount of amino acid content compared to cow and sheep cheese [6]. Goat cheese has high amount of Thr, Val, Lys and Ile [6]. Buffalo cheese is rich in essential amino acids such as Val, Lys, and Leu [7]. While in cow cheese the main amino acids are Glu, Pro, Lys and Leu [6]. Among the amino acids, branched-chain amino acids (e.g. leucine, isoleucine, and valine) are especially important because of their beneficial health effects [8] such as regulating blood sugar levels or protecting cells from oxygen-based damage [9]. This group of amino acids can be found in high amount in buffalo cheese [10].

During cheese making proteolysis is the main process that releases free amino acids (FAA), which play a key role in the nutritional quality of cheese. Beyond their nutritional significance, they also serve as key contributors to the development of cheese flavour, playing an important role in developing the distinct and desirable taste profiles of cheese [11]. FAAs are also known for their role in the formation of biogenic amines, as improper handling or aging of cheese can lead to the accumulation of these compounds, which can have adverse health implications in sensitive people.

The fatty acid composition also plays an important role in cheese characterization. The most abundant fatty acids detected in cheese are saturated palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). From the unsaturated fatty acids, the oleic acid (C18:1) and conjugated linoleic acid (C18:2) occur in higher amounts and have the potential to improve long-term health [12-15]. The levels of monounsaturated fatty acids (MUFA) are higher in cow cheese compared to cow and goat cheese [16]. While the levels of polyunsaturated fatty acids (PUFA) especially n3-PUFAs are higher in goat, which makes those cheeses better nutritional quality [6,16-18]. Buffalo cheese has high content of conjugated linoleic acid [19].

Aim of the Study

The aim of this study was to determine the nutritional quality of soft cheeses produced from the milk of three different animals using self-fermentation.

Materials and Methods

Cheese manufacture

Soft cheeses produced in the traditional way were provided by three local cheese manufacture of Kosovo. Soft cheeses were prepared from unpasteurized cow, goat and buffalo milk using self-fermentation for curd production as explained by Bytyqi, *et al*. [20]. Briefly, unpasteurized milk (8 - 10 kg) was placed in a container and was left to self-ferment for two days. After the self-fermentation, the cream on the surface of the curd was removed, and the curd was thermally treated for 30 minutes at 40°C. The curd was cut to release the whey, then it was dried for 12 hours at room temperature followed by pressure for 3 - 4 hours using a heavy pot and salting in brine. Three parallel samples were prepared (500g each).

Moisture and protein content determination

Moisture content was determined according to the ISO1442:1973 standard. Protein content was determined by the Kjeldahl method [21].

Amino acid determination

Protein-building amino acids

To determine protein-building amino acids, 500 mg of cheese samples were hydrolysed in a closed hydrolysing vessel (KUTESZ, Budapest, Hungary) with 10 mL 6 mol L^{-1} HCl in a nitrogen atmosphere at 110°C for 24 hours using a block thermostat (FALC Instruments, Treviglio, Italy). Neutralization was carried out in a 25 mL volumetric flask by first adding 10 mL of 4 mol L^{-1} NaOH to the hydrolysed sample and then filling the flask with sodium citric buffer (pH 2.2). Through a 0.25 µm membrane filter (Nalgene, Rochester, USA), the neutralized samples were filtered. An Automatic Amino Acid Analyzer AAA400 (Ingos Ltd., Prague, Czech Republic) equipped with a cation-exchange column (Ionex Ostion LCP5020 22 x 0.37 cm) was used to separate amino acids using stepwise gradient elution with sodium buffer systems. Colorimetric detection was done at 570 nm and 440 nm (for Pro) following post-column derivatization with a ninhydrin reagent. For the analysis, two parallel of samples for each type of cheese were prepared.

Free amino acids

To determine free amino acids (FAA), 3 g of cheese was extracted with 10 mL of 10% trichloroacetic acid using a Laborshake (Gerhardt GmbH, Königswinter, Germany) for an hour at ambient temperature at 100 rpm. After extraction, samples were twice filtered: once through filter paper and then through a 0.22 µm membrane filter (Nalgene, Rochester, USA). The same equipment was used for the determination of FAA as for protein-building amino acid analysis, along with the same detection procedure; however, lithium buffer systems were used for the stepwise gradient separation.

Fatty acid determination

Cheese samples of 1.0g were placed in a glass stoppered test tube, agitated with 10g of anhydrous sodium sulfate, then 40 mL of petroleum ether was added, and extracted for 6 hours using a Soxhlet extractor. The extraction step was followed by a solvent removal step (evaporation at 40°C, under an N_2 stream).

Preparation of fatty acid esters

To prepare the fatty acid esters the extracted fat was re-dissolved of in a 10 mL volume of n-hexane, using a Vortex mixer. Subsequently, 4 mL of a methanol solution containing 4% (w/v) potassium hydroxide (KOH) was pipetted into the solution and vigorously stirred for 5 minutes using the Vortex mixer. After phase separation, the upper iso-octane layer was entirely transferred to a clean test tube via a Pasteur pipette. To this iso-octane layer, 4 mL of distilled water was added and vortexed vigorously for 2 minutes. Then, 100 - 500 µL was transferred from the upper iso-octane phase, for gas chromatography measurement ensuring the exclusion of any aqueous phase, and then the sample vessel was sealed. All samples were stored at -18°C until used for gas chromatography analysis.

Gas chromatography

For the qualitative and quantitative analysis of fatty acid methyl esters (FAMEs) gas chromatography with flame ionization detection (GC-FID, Thermo Finnigan Trace GC, AS 2000 sampler, San Jose, CA, USA) was used. The separation took place on an SP2450 column (30m × 0.32 mm × 0.2 µm) with nitrogen (N_2) as the carrier gas, flowing at 0.5 $mL^{-}min^{-1}$, and a split ratio of 1:50. The injector temperature was held at 180°C. The heating temperature program was initiated with a 2-minute hold at 70°C, followed by a gradual increase to 140°C at a rate of 4°C min^{-1}, and then to 180°C at a rate of 1°C min^{-1}, each with a 1-minute hold. Finally, the temperature rose to 250°C at a rate of 40°C min^{-1}, with a 1-minute hold. The detector operated at 280°C.

Gas chromatography coupled with mass spectrometry (GC-MS, Hewlett Packard 5890/II GC and a 5971A MSD from Palo Alto, CA, USA.) was used to confirm component identification. An Ultra2 column (50m × 0.2 mm × 0.33 µm) was used for the separation, with helium as the carrier gas at a flow rate of 0.8 mL min^{-1} and a split ratio of 20:1. The sample was injected into a 250°C injector. The oven temperature program was initiated with a 2-minute hold at 70°C, followed by an increase to 280°C at a rate of 4°C min^{-1} for 10 minutes. The detector's ion source was set at 280°C, utilizing electron impact ionization with an electron energy of 70 eV. Mass spectra were collected in the range of 40 - 350 amu. The results were evaluated using the HP MS Chemstation program.

Statistical analysis

The statistical analysis was performed by IBMSPSS25 software [Gorge and Mallery, 2009]. To compare the quality parameters of cheeses produced from different milks, Multivariate Analysis of Variance (MANOVA) was used at $p < 0.05$. The normality of the differences was accepted by the Shapiro-Wilk and D'Agostino tests. Principal component analysis (PCA) was performed using XLSTAT Software (XLSTAT, 2022, Addinsoft) to categorize soft cheese samples based on their protein-building amino acids, free amino acids, and fatty acids composition.

Results and Discussion

Moisture and protein content of soft cheeses

The moisture and protein content of the soft self-fermented cheeses produced from buffalo, cow and goat milk was within the standards of the Joint FAO/WHO requirements [22] for standardized soft cheese with a minimum of 67% water. Moisture content was the highest in cow soft cheese (CC 77.50% ± 0.50), followed by buffalo cheese (BC 77.35% ± 0.60); goat cheese (GC 72.74% ± 0.30). The protein content was the highest in GC (19.79% ± 0.05), followed by CC (17.13% ± 0.15), and BC (10.72% ± 0.06). The protein content of goat cheese samples was higher compared to the other results reported by Rahayu, *et al*. [23] and Setyawardani, *et al*. [24] for goat cheeses (15.67% and 10.76%, respectively). Mustafa, *et al*. [25] found a lower (14.57%), while El Owin, *et al*. [26] found a higher protein content in cow cheese (22.50%) then we found in cow cheese samples. In the case of buffalo cheese, Shahein, *et al*. [27] detected higher (14.40%) protein content than was found in our samples. The low protein content of buffalo cheese can be explained by the fact that buffalos in Kosovo are bred for higher fat production.

Protein-building amino acid composition of soft cheeses.

Table 1 shows the amino acid (AA) composition of soft cheeses. Based on statistical analysis (MANOVA), there were significant differences ($p < 0.05$) in the amino acid composition of the three types of cheese (Table 1). The amino acid content was the highest in goat cheese (213.47 mg g^{-1}), followed by cow cheese (185.08 mg g^{-1}), while buffalo cheese had half amount of AA (106.68 mg g^{-1}). Of the 17 amino acids detected in soft cheese, the main amino acids were glutamic acid (BC 24.95%; CC 24.88%; GC 24.01%), proline (GC 17.12%; CC 16.10%; BC 15.06%), leucine (BC 9.87%; GC 9.04%; CC 8.74%), lysine (CC 6.73%; BC 6.19%; GC 5.71%) and aspartic acid (CC 6.14%; GC 5.66%; BC 5.65%). Goat and cow cheeses showed similar amino acid compositions. Buffalo cheese contained significantly higher amounts of methionine and a lower amount of histidine.

Essential amino acids accounted for approximately 40% (BC 39.48%; GC 37.60%; CC 35.98%) of the total amino acid content, making these soft cheeses a great source of proteins with a high nutritional value (Table 1). Although buffalo cheese has the lowest protein content (11%), its protein quality tends to be better than that of goat and cow cheese because its ratios of essential amino acids are higher than those of goat and cow cheeses.

Amino acids	Buffalo cheese		Cow cheese		Goat cheese	
	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%
Glutamic acid	27.21 ± 1.03	24.95^{a}	46.04 ± 1.41	24.88^{b}	51.26 ± 4.24	24.01^{b}
Proline	16.42 ± 1.00	15.06^{a}	29.80 ± 1.51	16.10^{ab}	36.55 ± 5.13	17.12^{b}
Leucine*	10.76 ± 1.22	9.87^{a}	16.17 ± 0.16	8.74^{b}	19.31 ± 2.18	9.04^{ab}
Lysine*	6.75 ± 0.17	6.19^{a}	12.45 ± 0.76	6.73^{a}	12.20 ± 0.47	5.71^{b}
Aspartic acid	6.17 ± 0.43	5.65^{a}	11.37 ± 0.06	6.14^{b}	12.08 ± 1.30	5.66^{a}
Valine*	5.64 ± 0.09	5.17^{a}	8.80 ± 0.34	4.75^{b}	12.98 ± 1.06	6.08^{c}
Serine	5.38 ± 0.27	4.94^{a}	9.76 ± 0.23	5.28^{a}	10.79 ± 1.00	5.06^{a}
Phenylalanine*	4.23 ± 0.10	3.88^{a}	7.47 ± 0.07	4.04^{b}	9.57 ± 0.31	4.48^{b}
Threonine*	4.07 ± 0.06	3.73^{a}	6.45 ± 0.22	3.49^{a}	8.46 ± 1.26	3.96^{a}
Tyrosine	4.06 ± 0.06	3.72^{a}	8.82 ± 0.65	4.77^{b}	9.84 ± 0.31	4.61^{b}
Isoleucine*	3.63 ± 0.06	3.33^{a}	5.04 ± 0.05	2.72^{b}	6.19 ± 0.69	2.90^{b}
Methionine*	3.05 ± 0.07	2.80^{a}	0.98 ± 0.01	0.53^{b}	1.03 ± 0.04	0.48^{b}
Arginine*	2.84 ± 0.06	2.60^{a}	5.18 ± 0.70	2.80^{a}	5.40 ± 0.21	2.53^{a}
Alanine	2.50 ± 0.11	2.30^{a}	4.63 ± 0.04	2.50^{a}	4.17 ± 0.38	1.95^{b}
Cysteine	2.40 ± 0.25	2.20^{a}	4.42 ± 0.94	2.39^{a}	5.48 ± 0.92	2.57^{a}
Histidine*	2.10 ± 0.05	1.92^{a}	4.04 ± 0.55	2.18^{b}	5.13 ± 0.31	2.40^{b}
Glycine	1.87 ± 0.10	1.71^{a}	3.65 ± 0.10	1.97^{a}	3.04 ± 0.44	1.43^{a}
Total Amino Acid content	109.08	100.00	185.08	100.00	213.47	100.00
Essential Amino Acid content	43.06	39.48	66.59	35.98	80.26	37.60

Table 1: *Protein-building amino acid composition of different soft cheeses.*

*Data are shown as mean ± standard deviation; n = 9; *Essential amino acids; [a,b,c]different letters indicate statistically significant differences ($p < 0.05$).*

Principal component analysis (PCA) was applied to the data to explore the distribution of the samples according to cheese type. Two principal components (PC) accounted for 97.58% of the total variance: PC1 and PC2 accounted for 92.37% and 5.21% of the total variance, respectively (Figure 1). PCA formed separate groups of cheeses based on the amino acid composition. The buffalo cheese was located on the negative side of PC2 (Figure 1). The differences between cow and goat cheese amino acid composition are less than compared to buffalo cheese. The buffalo cheese contained less amount of Glu and Pro and a much higher amount of Met.

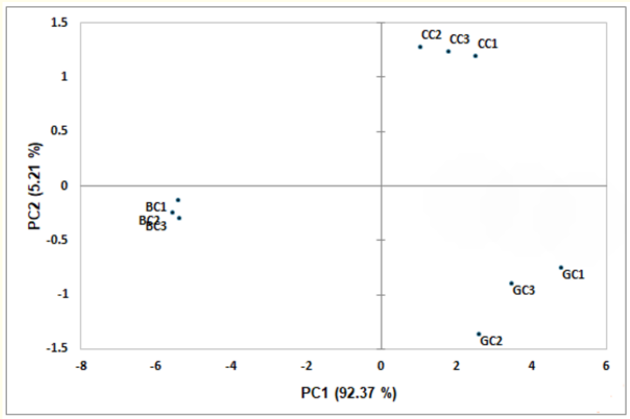

Figure 1: *Classification of soft cheeses by principal component analysis based on the protein-building amino acid composition.*

Free amino acid composition of soft cheeses

In addition to protein-building amino acids, the composition of free amino acids (FAA) in food products is also important in terms of quality. The composition of FAAs in cheese is affected by the production technology. The use of different starter cultures contributes to the development of free amino acids in cheeses. These are one of the main factors affecting the taste of cheeses [28,29]. Our results showed that even though no starter culture or rennet was added during cheesemaking, the concentration of free amino acids was relatively high (Table 2).

The main free amino acids found in buffalo cheese were GABA, Glu, Lys, Pro, and Asp, accounting for 61.63% of the total free amino acids (TFAA). In Goat cheese, GABA, Leu, Glu, Ala, and Pro were the main amino acids, with 59.28% TFAA. While in cow soft cheeses GABA, Lys, Pro, Met, Orn and Ala were the main FAA and represented 61.99% of the TFAA.

Buffalo cheese had higher amount of Glu (12.77%) and Asp (7.09%) than cow (Glu 2.64%; Asp 4.46%) and goat cheese (Glu 9.62%; Asp 3.66%). The GC had the highest amount of Leu (16.64%), Phe (5.38%), and Thr (3.63%) compared to BC (Leu 3.59%; Phe 1.99%; Thr 1.19%) and CC (Leu 32.13%; Phe 2.93%; Thr 1.08%). While the amounts of Lys (2.16%), Ser (1.50%), Tyr (0.54%) and Cys (0.16%) were lower in goat cheese compared to BC (Lys 12.79%; Ser 2.65%; Tyr 2.19%; Cys 2.29%) and CC (Lys 12.66%; Ser 3.72%; Tyr 3.66%; Cys 2.46%). Cow cheese shows significantly higher content of Met (7.13%) and Orn (6.92%), than BC (Met 4.62%; Orn 1.86%) and GC (Met 5.14%; Orn 0.78%).

Among free amino acids, GABA plays several roles in the human body, including hypotensive effects and relaxation, enhancement of immunity under stress conditions, inhibition of chronic diseases associated with alcohol prevention of cancer cell proliferation, prevention of diabetic conditions, and reduction of blood cholesterol levels [9,30]. GABA concentration was high in all cheese samples (CC 20.82%; BC

19.84%; GC 18.29%). Considering that 50% of all cheeses produced in Kosovo are self-fermented soft cheeses [20], daily consumption of these cheeses will contribute to their beneficial effects on health.

Branched-chain amino acid (BCAA: leucine, isoleucine, and valine) content was highly different in soft cheeses. Goat cheese had double the amount of BCAA (24.92%) than cow cheese (10.06%) and three times higher than buffalo cheese (8.75%). The BCAA plays a role in many physiological mechanisms such as regulating blood sugar levels, helping to lower the risk of oxygen-based damage to cells, maintaining tissue growth, and reducing fatigue during exercise [9,31]. The observed differences in BCAA content indicated that the nutritional composition of cheese strongly depends on the type of milk.

Soft cheeses had a high concentration of aromatic amino acids (AAA: phenylalanine and tyrosine), which is best known for their role in the nervous system [9]. The highest amount of AAA was detected in CC (6.59%), followed by GC (5.92%) and BC (4.18%).

Soft cheeses are a good source of sulfur-containing amino acids (Met+Cys: CC 9.59%; BC 6.91%; GC 5.30%), which serve important biological functions. Methionine and cysteine maintain normal cellular functions and health and are known for their antioxidant effect [32]. In countries with a plant-based diet, especially those with high consumption of legumes (e.g. Kosovo), consumption of soft cheese in addition to legumes helps satisfy the Recommended Dietary Allowance (RDA) for sulfur-containing amino acids (SAA), because the amount of SAA in legumes is very low [33].

Principal Component Analysis (PCA) showed good separation of cheese based on the free amino acid composition. Two principal components (PC) accounted for 95.52% of the total variance: PC1 73.39%; PC2 22.13% of the total variance (Figure 2). Goat cheese is located in the negative on the negative side of PC2. These differences may be caused by different levels of Leu, Phe and Asp.

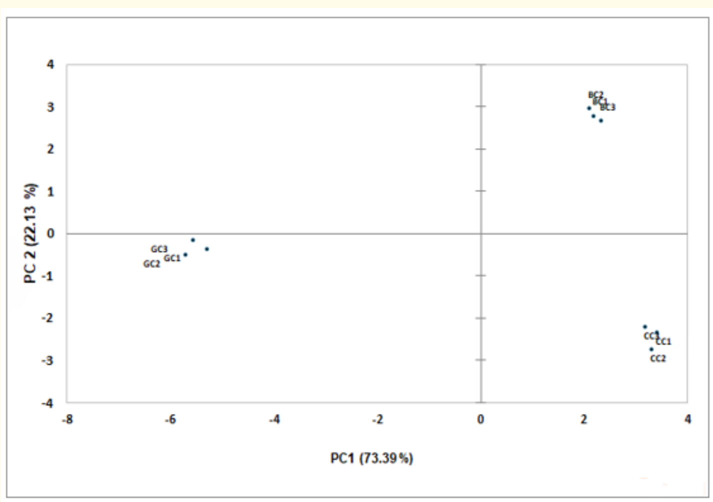

Figure 2: *Classification of soft cheeses by principal component analysis based on free amino acid composition.*

Free Amino Acids	Buffalo cheese	Cow cheese	Goat cheese
	%	%	%
Gamma-aminobutyric acid	19.84[a]	20.82[b]	18.29[b]
Leucine*	3.56[a]	2.13[a]	16.64[b]
Glutamic acid	12.77[a]	2.64[b]	9.62[c]
Proline	9.34[a]	9.57[a]	6.79[b]
Alanine	2.99[a]	6.73[b]	6.61[b]
Methionine***	5.62[a]	7.13[b]	5.14[a]
Phenylalanine**	1.99[a]	2.93[a]	5.38[b]
Valine*	2.46[a]	4.22[b]	5.21[b]
Asparagine	3.61[a]	1.21[b]	4.17[a]
Threonine	1.19[a]	1.08[a]	3.63[b]
Histidine	3.82[a]	1.40[b]	3.24[a]
Aspartic acid	7.09[a]	4.46[b]	3.22[b]
Isoleucine*	2.73[a]	3.71[b]	3.16[b]
Glutamine	0.79[a]	0.37[a]	2.98[b]
Lysine	12.79[a]	12.66[a]	2.16[b]
Serine	2.65[a]	3.72[a]	1.50[b]
Ornithine	1.86[a]	6.92[b]	0.78[c]
Glycine	0.40[a]	0.78[a]	0.82[a]
Tyrosine**	2.19[a]	3.66[a]	0.54[b]
Cysteine***	1.29[a]	2.46[a]	0.16[b]
Cystathionine	1.02[a]	1.41[a]	ND
SUM	100.00	100.00	100.00
Branched Chain Amino Acids	8.75[a]	10.06[b]	24.92[c]
Aromatic Amino Acids	4.18[a]	6.59[b]	5.92[c]
Sulphur-containing Amino Acids	6.91[a]	9.59[b]	5.30[c]

Table 2: *Free amino acid composition of soft cheeses.*

**Branched-chain Amino acids: **Aromatic Amino Acids: ***Sulphur-containing Amino Acids); [a,b,c]different letters show statistically significant differences ($p < 0.05$).*

Fatty acid composition of soft cheeses

Table 3 shows the fatty acid profiles of the soft cheeses. The proportion of each fatty acid group in their profiles varied according to the type of milk used in cheesemaking. Among the identified fatty acids, the highest differences were observed in the presence of caprylic acid (C10:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). Goat cheese had the highest proportion of caprylic acid (9.63%), myristic acid (11.61%), and palmitic acid (30.47%), followed by cow cheese (1.24% C10:0; 3.33% C14: 0; 25.04% C16:0) and buffalo cheese (1.26% C10:0; 1.94% C14:0; 11.02% C16:0). In buffalo cheese, stearic acid (37.63%)

and linoleic acid (19.75%) were significantly higher than in cow cheese (19.61% C18:0; 3.15% C18:2) and goat cheese (10.82% C18:0; 3.32% C18:2). Cow cheese had the highest levels of oleic acid (33.39%), followed by goat cheese (19.14%), and buffalo cheese (10.51%).

Saturated fatty acids (SFA) were the main group in soft cheeses (Table 3). The highest amount were found in goat cheese, accounting for 74.45% of the total fatty acid content, followed by buffalo cheese (60.11%) and cow cheese (54.80%). The results were similar to those reported by Pajor, *et al*. [34] and Van Nieuwenhove, *et al*. [18] for goat cheese (71.40 - 73.40% and 69.10%, respectively). SFA contents of cow and buffalo cheeses were lower than those reported by Prandini, *et al*. [35] for cow cheese (65.23 - 68.52%) and Van Nieuwenhove, *et al*. [19] for buffalo cheese (65.50%).

Among the unsaturated fatty acid groups, monounsaturated fatty acids (MUFA) were the highest in cow cheese (37.25%), followed by goat cheese (21.08%), and buffalo cheese (13.66%). The MUFA content of goat and cow cheeses was similar to that reported in the literature, namely 22.80 - 24.30% in goat cheese [34] and 27.90-31.19% in cow cheese [35]. The MUFA content of buffalo cheese was much lower than that reported (31.80%) by Van Nieuwenhove, *et al*. [19].

Buffalo cheese had an order of magnitude higher polyunsaturated fatty acid (PUFA) content (26.60%) than goat (3.96%) or cow cheese (7.51%). Linoleic acid was the main PUFA in the buffalo cheese (19.75%). The amount of PUFA in buffalo is not only higher than the PUFA content of cow and goat cheeses but also higher than that reported earlier (2.60%) in buffalo cheese [19]. The high amount of PUFA in buffalo soft cheese indicate the good fat quality of this Kosovan buffalo breed.

Fatty acids	Buffalo cheese (%)	Cow cheese (%)	Goat cheese (%)
Butyric acid C4:0	1.55^{a}	2.46^{b}	1.51^{a}
Caproic acid C6:0	2.15^{a}	1.40^{b}	1.73^{b}
Capric acid C8:0	2.24^{a}	1.72^{b}	3.35^{c}
Caprylic acid C10:0	1.26^{a}	1.24^{a}	9.63^{b}
Lauric acid C12:0	2.32^{a}	ND	5.33^{b}
Myristic acid C14:0	1.94^{a}	3.33^{b}	11.61^{c}
Palmitic acid C16:0	11.02^{a}	25.04^{b}	30.47^{c}
Stearic acid C18: 0	37.63^{a}	19.61^{b}	10.82^{c}
Palmitoleic acid C16:1	2.55^{a}	3.86^{b}	1.94^{c}
Oleic acid C18:1	10.51^{a}	33.39^{b}	19.14^{c}
Linoleic acid C18:2	19.75^{a}	3.15^{b}	3.32^{b}
Alfa-linolenic acid C18:3	6.85^{a}	4.36^{b}	0.64^{c}
Unknown	0.23^{a}	0.44^{a}	0.51^{a}
Sum SFA	60.11^{a}	54.80^{b}	74.45^{c}
Sum MUFA	13.06^{a}	37.25^{b}	21.08^{c}
Sum PUFA	26.60^{a}	7.51^{b}	3.96^{c}

Table 3: *Fatty acid composition in soft cheeses.*

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ^{a,b,c}different letters show statistically significant differences ($p < 0.05$) ND: Not Detected.

The fatty acid data of the cheeses were evaluated using Principal Component Analysis (PCA) to examine the distribution of samples based on cheese type. The PCA revealed two principal components (PC) that explained a significant portion of the total variance, with PC1 and PC2 accounting for 52.24% and 40.37% of the total variance, respectively. These two components cumulatively explained 92.64% of the total variance, indicating their relevance in capturing the variation in fatty acid composition among the cheeses. The cow cheese was situated on the negative left side of the plot (Figure 3), while the buffalo cheese was located on the positive left side, and the goat cheese on the positive right side of the plot. The distinct clustering of the cheeses based on their fatty acid composition highlights the potential differences in their nutritional profiles and could provide valuable insights for further analysis and characterization.

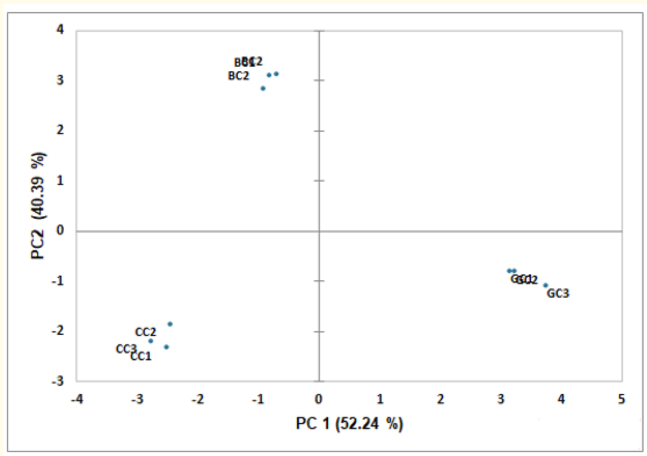

Figure 3: *Classification of soft cheeses by principal component analysis based on the fatty acid composition.*

Conclusion

The results of the study provide important information on protein-building amino acid, free amino acid, and fatty acid composition of soft cheeses made from unpasteurized buffalo, cow, and goat milk with self-fermentation. The results highlight the nutritional characteristics of each cheese type, with goat and cow cheeses being the best based on the essential amino acids and buffalo cheese on the bases of the essential fatty acid content. In addition to contributing to the knowledge of the nutritional value of different soft cheeses, our results provide useful information for consumers to choose cheese types based on their specific nutritional needs and preferences.

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Conflict of Interest

The authors declare no conflict of interest.

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