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Characterization of traditional foods of Kosova based on amino acid, biogenic amine and fatty acid composition

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LIST OF ABBREVATIONS

AAA- Aromatic Amino Acids

ANOVA- Analysis of Variance

BA- Biogenic Amines

BC- Buffalo cheese

BCAA- Branch Chain Amino Acids

CC- Cow Cheese

EAA- Essential Amino Acids

FAA- Free Amino Acids

FAMEs- Fatty Acid Methyl Esters

FFA – Free Fatty Acids

FID- Flame Ionization Detection

GC- Goat Cheese

LAB-Lactic Acid Bacteria

LPL- Lipoprotein lipase

MANOVA- Multivariate Analysis of Variance

MUFA- Monounsaturated Fatty Acids

PUFA- Polyunsaturated Fatty Acids

RC- Rugova Cheese

SAA- Sulphur containing Amino Acids

SC- Sharri Cheese

SFA- Saturated Fatty Acids

TAA- Total Amino Acid content

1. INTRODUCTION

Traditional foods in Kosovo represent an invaluable component of the country's cultural identity and nutritional heritage, having been consumed for centuries and passed down through generations. They embody a wealth of knowledge on food production, preservation, and consumption practices that remain integral to rural and urban communities alike. Beyond their cultural role, these foods are important dietary staples that contribute to the nutritional well-being of the population. Among their key constituents are amino acids, biogenic amines, and fatty acids, which not only define the sensory qualities of food but also determine their potential impact on human health. While amino acids serve as the building blocks of proteins and are critical for muscle development, neurotransmitter synthesis, and metabolic functions, free amino acids also enhance taste and aroma. Biogenic amines, though naturally occurring and essential for physiological processes, can pose risks when present at elevated concentrations. Likewise, fatty acids influence both the nutritional quality and sensory profile of foods, with an optimal balance of saturated and unsaturated fatty acids contributing to health-promoting effects.

Despite their importance, systematic scientific data on the nutritional composition of traditional Kosovan foods remain scarce. The Busha cattle breed, for example, is endangered, and its milk composition had not been studied in detail prior to this work, despite its significance for both cultural preservation and nutritional diversity. Similarly, while traditional cheeses such as Sharri and Rugova are widely consumed and culturally valued, there has been little documentation of their amino acid, fatty acid, or biogenic amine composition. This gap in knowledge extends to traditional beef products, including dry meat, ham, and homemade sausages, which are consumed regularly yet lack scientific evaluation of their nutritional value and potential food safety risks. Without such data, it is difficult to assess their role in supporting dietary quality, protecting consumer health, or promoting their wider marketability.

The COVID-19 pandemic further underscored the importance of resilient local food systems and traditional production methods, which can provide food security during times of crisis. In this context, a deeper understanding of the nutritional composition and safety of traditional Kosovan foods is of both scientific and practical relevance. By analyzing Busha milk, traditional cheeses, and traditional beef products, this study aims to generate the first comprehensive nutritional database for Kosovo, addressing both the health benefits and potential risks associated with their consumption. The outcomes of this research not only enhance consumer awareness and scientific knowledge but also support cultural heritage preservation, conservation of endangered livestock breeds, and the development of food safety and quality guidelines.

2. OBJECTIVES

The study of Kosovan traditional food composition was undertaken with the dual aim of preserving cultural heritage and providing a scientific basis for evaluating the nutritional potential of these foods. Traditional foods in Kosovo, such as Busha cattle milk, homemade cheeses, and beef products, represent centuries-old dietary practices that have not yet been systematically characterized. Given their wide consumption and cultural significance, the main objective of this research was to establish a comprehensive nutritional profile of these products, focusing on amino acid composition, biogenic amine content, and fatty acid profiles. Such an evaluation is not only scientifically important but also necessary for enhancing consumer knowledge, supporting public health initiatives, and strengthening the marketability of traditional foods.

A particular emphasis of the work was placed on Busha cattle milk, as this breed is endangered and its unique milk composition had not been analyzed. The objective here was to determine its amino acid profile, biogenic amine levels, and fatty acid composition, and to evaluate its nutritional quality in relation to international dietary standards. By doing so, the study aimed to provide evidence for the potential value of preserving the Busha breed, not only as a cultural and genetic resource but also as a contributor to dietary quality and food diversity in Kosovo.

Another key objective of the research was to analyze traditional cheeses, including those made from cow, goat, and buffalo milk as well as the region-specific Sharri and Rugova varieties. The nutritional analysis focused on essential and free amino acids, biogenic amines, and fatty acid profiles, with the aim of identifying their health-promoting properties. By comparing the nutritional composition across cheese types, the study sought to determine which varieties are superior sources of essential nutrients, and at the same time to identify potential food safety concerns related to biogenic amine accumulation. This approach was expected to provide valuable insights into both the benefits and risks of consuming these culturally significant dairy products.

The final objective was to examine traditional beef products such as dry beef meat, beef ham, and homemade sausages. These products form an integral part of the Kosovan diet, yet little is known about their precise nutritional value. Through detailed analysis of their amino acid composition, biogenic amine content, and fatty acid profiles, this research aimed to compare them with both established nutritional standards and commercially available products. The goal was to highlight their role as potential sources of high-quality protein and essential fatty acids, while also assessing potential health risks associated with biogenic amines. Together, these objectives were designed to produce the first comprehensive nutritional database of traditional Kosovan foods, thereby contributing to scientific knowledge, consumer awareness, and the preservation of cultural food heritage.

3. LITERATURE REVIEW

3.1. Traditional food products

Traditional food products are vital to the cultural, nutritional, and economic fabric of societies. The conceptualization of traditional food has evolved over time, encompassing dimensions such as time, place, know-how, and cultural meaning (Rocillo-Aquino et al., 2021). These foods are deeply rooted in the heritage of communities and are often transmitted through generations, preserving unique culinary practices and cultural identities (Peulić et al., 2023). The historical development of food systems reveals that traditional foods have been essential for societal sustenance and cultural continuity. Traditional foods embody the accumulated knowledge and practices related to food production, preparation, and consumption, which are passed down across generations (González & García Álvarez, 2023; Rocillo-Aquino et al., 2021). This transmission of knowledge ensures the preservation of cultural heritage and the continuity of traditional agricultural and culinary practices (Rocillo-Aquino et al., 2021).

Traditional foods are also crucial in promoting human and environmental health. Traditional dietary patterns, which often include a diverse array of locally sourced ingredients, enhance nutritional security and agricultural resilience (Deaconu et al., 2021). For instance, traditional foods in Indigenous communities in Ecuador are linked to improved nutritional outcomes and sustainable agricultural practices (Deaconu et al., 2021). Nutritional benefits of traditional foods are well-documented, particularly those involving fermented foods. These foods contribute to gut health and overall well-being (Negrete-Romero et al., 2021). Traditional diets, such as those of Aboriginal populations rich in omega-3 fatty acids, show how this food practices can positively impact health by providing essential nutrients that modern diets often lack (Negrete-Romero et al., 2021; Peulić et al., 2023)

Consumer attitudes towards traditional food products emphasize qualities such as authenticity, heritage, and the use of natural ingredients. A study by (Peulić et al., 2023) showed that 98.3% of the respondents consume traditional food products in their households. The specific characteristics that consumers prioritize when buying food products are products with no added sugar (41.2%), traditional production processes (38.8%), GMO-free products (36.9%), organic products (36.4%), and additive-free products (34.9%). There is a significant consumer preference for traditional foods due to their perceived health benefits, quality, and cultural value (Peulić et al., 2023). Certification plays a vital role in ensuring product quality and meeting consumer demands for health and nutrition, further boosting consumer confidence and preference for traditional food products (Peulić et al., 2023).

The preservation of traditional foods is crucial for maintaining their availability and nutritional value. Traditional methods like curing, pickling, and smoking are still valued for their ability to enhance flavor and longevity. Modern techniques, such as pasteurization, freeze drying, and vacuum packing, have been integrated to meet contemporary food safety standards and consumer preferences (Kumar, 2019). These methods ensure that traditional foods remain relevant and accessible in modern markets.

Traditional food products are not merely remnants of the past but are dynamic components of modern dietary systems that contribute to cultural identity, nutritional well-being, and sustainable food practices. Their continued relevance and evolution highlight the importance of preserving and promoting these foods within the global food system. By valuing and integrating traditional food practices, societies can enhance cultural heritage, improve health outcomes, and support sustainable agricultural practices.

3.2. Milk and its composition.

Milk is a complex biological fluid widely recognized for its rich and diverse composition, which is naturally adapted to meet the nutritional requirements of mammalian offspring. Comprising approximately 87% of water, milk serves as a vital source of hydration. Milk's composition is critical to understanding its various health benefits and applications, as it is one of the most consumed and versatile foods in the world. The chemical composition of milk varies greatly depending on the species of the animal from which it is derived, with cow's milk being the most consumed type by humans.

Milk's key components include water, proteins, fats, lactose, vitamins, and minerals, all of which contribute to its overall nutritional value (Vaskova et al., 2016). Milk proteins, including casein and whey proteins, are a rich source of essential amino acids. Casein, which forms about 80% of milk proteins, contributes to milk's white, opaque appearance and provides a slow-release source of amino acids[4]. Whey proteins, on the other hand, are soluble proteins that offer diverse bioactive properties, such as immune modulation and antioxidant effects (Aqib et al., 2019; Gorissen et al., 2018; Karimova & Ismailova, 2023; Roy et al., 2020). A study by (Rafiq et al., 2016a) highlighted the potential of these proteins in promoting muscle synthesis and recovery after exercise. Triglycerides, the main lipid component in milk, are present within milk fat globules dispersed throughout the aqueous phase (Karoui et al., 2010). Milk fat contributes to the creamy texture and flavor of dairy products, while also serving as a concentrated source of energy and essential fatty acids (Aqib et al., 2019; Roy et al., 2020; Vaskova et al., 2016). Recent studies have shown that milk fat contains bioactive lipids, such as conjugated linoleic acid and omega-3 fatty acids, which have been associated with various health benefits, including anti-inflammatory and anticancer properties (Basak & Duttaroy, 2020; Belury, 2023a; Dilzer & Park, 2012a). Lactose, the primary carbohydrate in milk, is a disaccharide composed of glucose and galactose. It serves as a readily available energy source and facilitates the absorption of calcium and other minerals. Lactose also plays a role in the development of the gut microbiota, which is crucial for overall health (Aqib et al., 2019; Karimova & Ismailova, 2023; Roy et al., 2020; Yusuf Bekere & Husen, 2020). Milk is a rich source of vitamins and minerals essential for human health. Vitamin A promotes vision and immune function, vitamin D supports calcium absorption and bone health, and B vitamins play crucial roles in energy metabolism and nervous system function. Minerals such as calcium, phosphorus, potassium, and magnesium contribute to bone health, muscle function, and electrolyte balance (Aqib et al., 2019; Karimova & Ismailova, 2023; Mohr et al., 2023; Roy et al., 2020; Yusuf Bekere & Husen, 2020).

The composition of milk varies widely among different mammalian species (Table 1) due to genetic, physiological, nutritional factors, and environmental conditions (Bekele et al., 2023; Lindmark-Månsson et al., 2003; Moschovas et al., 2023; Schönfeldt et al., 2012). Cow milk has a high fat content, with an average fat-to-protein ratio of about 2:1. Buffalo milk has a higher casein-to-protein ratio, making it suitable for cheese making(S. Ahmad et al., 2008; FAO, 2024). Camel milk is similar but slightly saltier, rich in vitamin C and unsaturated fatty aci Sheep milk has higher fat and protein contents than goat and cow milk, with only buffalo and yak milk containing more fat. Sheep milk is particularly suitable for cheese and yoghurt making, especially in the Mediterranean region. Goat milk has a similar composition to cow milk, with some countries transforming it into cheese while others consume it raw or acidified (FAO, 2024; Park et al., 2007). Yak milk has a sweet taste and fragrant smell, with between 15-18% solid content, 5.5-9% fat, and 4-5.9 percent protein. It is used mainly by herders and their families in milky tea and can be processed into various milk products like butter, cheese, and fermented milk (FAO, 2024).

Table 1. Milk composition of different animals (g/100ml)

Milk composition		Cattle	Sheep	Goat	Buffalo
Solid	Total	11.8-13.0	18.1-20.0	11.9-16.3	15.7-17.2
	Protein	3.0-3.9	4.5-7.0	3.0-5.2	2.7-4.7
	Fat	3.3-5.4	5.0-9.0	3.0-7.2	5.3-9.0
	Lactose	4.4-5.6	4.1-5.9	3.2-5.0	3.2-4.9
	Ash	0.7-0.8	0.8-1.0	0.7-0.9	0.8-0.9

*Source: Adapted and modified from (Roy et al., 2020)

Milk is a vital food that plays a significant role in human nutrition and health, serving as a cornerstone of balanced diets across the lifespan. It contains essential nutrients such as proteins, peptides, lipids, vitamins, and minerals, which exhibit diverse physiological functions with potential health benefits. For instance, whey proteins contain bioactive peptides with antimicrobial, antioxidant, and immune-modulating properties, while milk fat globule membrane components contribute to intestinal health and lipid metabolism regulation(Auestad & Layman, 2021). In the dairy industry, knowledge of milk composition is essential for optimizing production processes, ensuring product consistency and quality, and meeting consumer demand for nutritious and safe dairy products (Suglobov & Khabipov, 2022). By understanding factors influencing milk composition, dairy farmers and processors can implement strategies to enhance milk quality, such as optimizing animal nutrition, genetics, and management practices.

Cow milk is the most consumed type of milk globally, containing 18 of 22 essential nutrients, including various bioactive peptides and fatty acids (Cimmino et al., 2023). Sheep's milk is a rich source of essential nutrients, including high-quality proteins, vitamins, and minerals. It is an important source of bioactive substances with health-promoting functions for the body, including antibacterial, antiviral, and anti-inflammatory properties(Flis & Molik, 2021). Buffalo milk, the second largest type of milk globally, contributes approximately 13% to the world's total milk production (Khetra et al., 2022). Goat's milk and its products are an important source of fatty acids, calcium, phosphorus, iron, and magnesium (Thakur et al., 2024). Goat milk products have a health-promoting effect, especially on the cardiovascular system and civilization diseases. Consuming yogurt and kefir reduce obesity and the risk of metabolic syndrome and prevents type II diabetes. Goat's milk also contains many biologically active, antibacterial, immunomodulatory, and antioxidant substances (Thakur et al., 2024).

Busha cattle (*Bos primigenius f. taurus*) is a unique breed of cattle found on the Balkan Peninsula, with various varieties including those in Kosovo, Albania, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, and Macedonia. These breeds are known for their hardiness, adaptability to harsh environments, and low managerial input. They live on grass and bushes during summer and are fed with small amounts of hay, corn, or wheat from autumn until spring (Table 2). Bushas provides sufficient milk and meat for farmers and their families, ensuring autonomic survival (Krasniqi et al., 2013).

Despite being the dominant breed in most Balkan countries until the 1950s and 1960s, it has been replaced by more productive and specialized breeds in lowland areas where intensive farming is practiced (Bunevski et al., 2016). The Busha breed is known for its low production rate but high resistance against diseases and good adaptation to extensive breeding conditions. Living conditions for Busha cattle are mostly in hilly and mountain areas, where other breeds are bred. Busha cattle are characterized by good pasture utilization, resistance to diseases, and the possibility of breeding even under difficult conditions (Krasniqi et al., 2013). They have low production costs and environmental adaptability in Kosovo. Busha cattle feed mainly on pasture (250 days/year) consisting of *Gramine* plants followed by *Leguminosae,* while the rest of the year they are kept at stables for about 115 days/year (Bytyqi et al., 2013; Krasniqi et al., 2013). Small size breed Busha and its crosses tend to have lower somatic cells count content (SCC), which according to milk quality produced is in Class Extra (Bytyqi et al., 2013).

Dukagjini Busha and Sharri Busha are two types of Busha cattle in Kosovo (Table 2). Dukagjin Busha is characterized by higher production and bigger size animals compared to other

Busha strains. Sharri Busha, also known as Busha, Illyrian Cattle, Brachyceros, Rhodopi, and Rodopy Shorthorn, has a history dating back to the Neolithic age in the Balkans (Bytyqi, 2009).

Table 2. Characteristics of Busha cattle strains of Kosova

Details:	**Dukagjin Busha**	**Sharri Busha**
Area of distribution	Western part of Kosovo (Gjakova, Deqani, Istog, Peja and Klina), also Montenegro	Sharri mountains
Population size	Less than 1000	less than 1000
Risk status	Endangered	Endangered
Color	Intensive red, sometimes dark red	intensive red, sometimes yellowish, dark red, tiger
Head	Small, narrow, mug is dark coloured with some white or black hair around	Small, narrow, mug is dark coloured with some white hair around
Horns	Lyre shaped horns	Lyre shaped horns
Weight	340-380 kg	330-350kg
Height	100-120 cm	105-115 cm
Use	Milk, meat	Milk, meat
Productivity	1200-1800 l, <3.6% fat	900-1500 l, 3.5-5% fat
Remarks	No conservation programme, no breeding programme running	conservation programme in Prizren

*Source: Adapted from (Bytyqi, 2009)

3.2.1. Amino acid composition of milk protein

Milk proteins are chains of amino acid molecules connected by peptide bonds, each with its own unique sequence. There are 22 different amino acids, with 10 essential amino acids (leucine, lysine, valine, phenylalanine, tryptophan, threonine, isoleucine, methionine, arginine and histidine) that the human body cannot synthesize and must obtain from diet (Fondation de technologie laitière du Québec, 1985; Varnam & Sutherland, 2001). Milk proteins contain all essential amino acids required by humans.

Milk contains approximately 3.3% protein, which is synthesized in the mammary gland; however, 60% of the amino acids used in milk protein synthesis come from the animal's diet. Milk proteins are broadly classified into two types: caseins and whey proteins. Cow's milk contains approximately 82% casein and the remaining 18% serum, or whey protein. The casein protein includes α-s1, α-s2, ß, and κ caseine, while the whey proteins are alpha-lactalbumin, lactoferrin, beta-lactoglobulin, and glycomacropeptide. each with unique amino acid compositions (Fondation de technologie laitière du Québec, 1985; Varnam & Sutherland, 2001)

Total milk protein content and amino acid composition vary with cow breed and individual animal genetics. Milk proteins are crucial components of the human diet, contributing significant nutritional, biological, and functional properties. They play a pivotal role in human health, with milk protein composition affecting the nutritional and physical properties of dairy products. Caseins and whey proteins have a unique amino acid profile. These amino acids have unique roles in human metabolism, providing substrates for protein synthesis, suppressing protein catabolism, and serving as substrates for gluconeogenesis (Haug et al., 2007; Lapierre et al., 2020a; Mills et al., 2011).

Milk proteins stimulate insulin secretion, like the response seen after ingestion of whey. A combination of milk with a high glycaemic load may stimulate insulin release and reduce postprandial blood glucose concentration, potentially lowering the risk of diseases related to insulin resistance syndrome (Khaldi et al., 2014; Kim & Lee, 2021; Schwab & Broderick, 2017). Peptides encrypted in the primary amino acid sequences of proteins and released during digestion are increasingly recognized as biologically active protein metabolites that may have beneficial effects on human health. These peptides exhibit a wide range of bioactivities, including satiety, antimicrobial, mineral-binding, and anti-lipidemic properties (Kim & Lee, 2021; Lapierre et al., 2020b; Mills et al., 2011; Varnam & Sutherland, 2001). The synthesis of proteins in milk is a complex and high-cost process, with low conversion efficiency in dairy cows. However, research has shown that this can be increased using protein supplements or a single amino acid supply (Kim & Lee, 2021).

3.2.2. Biogenic amines in milk

Biogenic amines (BAs) are low molecular weight nitrogenous organic compounds with various biological activities, mainly formed by the decarboxylation of amino acids (Linares et al., 2011). They are naturally occurring organic nitrogen compounds, classified into aliphatic, aromatic, or heterocyclic groups. The formation of BA in food requires the availability of free amino acids, presence of decarboxylase-positive microorganisms, and favorable conditions for bacterial growth and decarboxylase activity (Simon Sarkadi, 2017). BA are present in a wide range of foods, including dairy products, and can accumulate in high concentration . They are essential for organisms and participate in various physiological processes, including cell proliferation, growth, differentiation, and neurotransmission (Bardócz, 1995; Silla Santos, 1996). They also affect protein synthesis and DNA and RNA biosynthesis. However, excessive BAs can have negative effects on human health, as they can be toxic in excessive quantities, especially histamine and tyramine, are risk factors for food intoxication and may cause unpleasant allergic symptoms (Linares et al., 2011; Ruiz-Capillas & Herrero, 2019; Simon Sarkadi, 2017; Wójcik et al., 2021).

In dairy products, the most important BAs are histamine, tyramine, putrescine, and cadaverine (Linares et al., 2011). Histamine and tyramine are produced by the enzymatic decarboxylation of histidine and tyrosine, while putrescine is synthesized via ornithine decarboxylation or agmatine deamination. Cadaverine originates from lysine decarboxylation. Fermented foods, including milk-based fermented foods like cheese, are often associated with biogenic amines (BA) poisoning. Cheese is the main dairy product likely to contain potentially harmful levels of BAs, especially tyramine, histamine, and putrescine. Despite the toxicity of BAs, their limit concentrations in fermented foodstuffs have not yet been adequately standardized by regulatory agencies (EFSA, 2011).

3.2.3. Fatty acid composition of milk fat

Milk fat plays a pivotal role in defining the physical and sensory attributes of dairy products. It is a complex mixture of lipids, predominantly composed of triacylglycerol (98%), with minor constituents including diacylglycerol, cholesterol, phospholipids, and free fatty acids. Other lipids present in milk fat encompass hydrocarbons, fat-soluble vitamins, and flavor compounds (Park et al., 2017; P. C. Pereira, 2014).

Saturated fatty acids constitute approximately 70% of the total milk fat, while unsaturated fatty acids account for the remaining 30%. Remarkably, milk fat comprises over 400 different fatty acids. The principal fatty acids in milk fat are oleic acid (29%), palmitic acid (26%), and stearic acid (14%) (El-Abassy et al., 2011; Mills et al., 2011; Varnam & Sutherland, 2001). Fatty acids (FAs) in milk fat are significant nutritional components in human diets and can impact human health. While previously viewed negatively, the perception of FAs' impact on health has shifted positively over the last decade (Lambrini et al., 2021; P. C. Pereira, 2014). The FA profile in dairy farming is crucial for the technological quality of raw milk and can enhance the value of dairy products

The fatty acid composition of milk fat is influenced by a myriad of factors such as the animal's origin, stage of lactation, health status (e.g., mastitis), ruminal fermentation, and dietary factors (Bekele et al., 2023; Lindmark-Månsson et al., 2003; Moschovas et al., 2023; Schönfeldt et al., 2012).. These factors contribute to the variability in the amount and composition of milk fatty acids.

3.3. Cheese

3.3.1. Cheese and its classification

Cheese is an ancient, fermented dairy product with a long history of production, dating back about 8,000 years, is believed to have evolved in the Fertile Crescent between the Tigris and Euphrates rivers in Iraq during the "Agricultural Revolution" (Foxx et al., 2017; Sandine & Elliker, 1970) Originating in West Asian countries, it is traditionally called "milk pimples" by nomadic people in northwestern China (Zheng, Ge, et al., 2021). Cheese production involves curdling milk, cream, or partially skimmed buttermilk from cow or goat (or a combination of these) and subsequently separating the whey. The process begins by adding an optimal quantity of lactic acid bacteria (LAB) starter and rennet to the milk, leading to transformations in milk proteins, carbohydrates, and fats. After removing the whey, the remaining product undergoes ripening for a specific duration (Foxx et al., 2017). According to the (FAO, 1978) cheese definition is:

> *"Cheese is the ripened or unripened soft, semi-hard, hard, or extra-hard product, which may be coated, and in which the whey protein/casein ratio does not exceed that of milk, obtained by:*
>
> > *a) coagulating wholly or partly the protein of milk, skimmed milk, partly skimmed milk, cream, whey cream or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from the coagulation, while respecting the principle that cheese-making results in a concentration of milk protein (in particular, the casein portion),*

and that consequently, the protein content of the cheese will be distinctly higher than the protein level of the blend of the above milk materials from which the cheese was made; and/or

b) processing techniques involving coagulation of the protein of milk and/or products obtained from milk which give an end-product with similar physical, chemical and organoleptic characteristics as the product defined under (a).”(FAO, 1978)

Cheese, a fermented dairy product with high nutritional value, is considered one of the pearls in the dairy industry (Kourkoutas et al., 2006). The consumer's preference for cheese is significantly influenced by its sensory attributes, particularly flavor and aroma. The distinctive qualities and exceptional nature of cheese arise from a complex interplay of compounds and molecules, such as fatty acids, amines, ketones, free amino acids, alcohols, aldehydes, lactones, and sulfur compounds (D’Incecco et al., 2020; Gatti et al., 2014; Zheng, Shi, et al., 2021). The presence of these molecules is linked to various cheese-making factors, including climate, regional conditions, geographical location, technological processes, the microbiota associated with cheese, and ripening conditions. Cheese flavor formation involves four key pathways: proteolysis, lipolysis, glycolysis, and citrate utilization. Beyond bacteria and mold, research indicates that *Geotrichum candidum* impacts carbohydrate, lipid, and amino acid metabolism, while *Debaromyces hansenii* specifically influences other amino acid metabolisms. The production of flavor compounds relies on the milk-degrading enzymes of each fermenting strain and the interplay of metabolic pathways among strains(Gatti et al., 2014; Sheikha, 2018; Zheng, Ge, et al., 2021; Zheng, Shi, et al., 2021).

Over 2,000 different cheese varieties exist, with over 400 more famous, which are classified according to different characteristics including: country or region of origin, type of milk, texture, maturation time, and fat content (McSweeney et al., 2004). The criteria which is used the most in cheese classification is texture (soft, semi-soft, semi- hard, hard and very hard) which is based in moisture content (McSweeney et al., 2004). According to Farkye, (2004) hard cheeses are firm or very firm in structure and require some form of pressure to break apart, they are characterized by upper limits for moisture content and lower limits for fat content. While the term soft cheese is used to describe cheese that is soft to touch or to pressure applied between fingers, soft cheeses must contain a minimum of 50% fat dry matter (Farkye, 2004).Cheese preservation and ripening methods can vary greatly depending on moisture content. Extra-hard cheeses, like Parmesan and Romano, are produced from very hard curds, while hard cheeses like cheddar have a 3-12 month ripening period. Semi-soft cheeses, like Limburger and blue cheese, are ripened using bacteria and mold. The variety of cheese produced is determined by raw milk characteristics, fresh curd preparation method, and microorganisms in the milk or curds. The types of microbes involved in cheese production or ripening are determined by inoculated microbes, cheese production conditions, and environmental factors (Foxx et al., 2017; Gatti et al., 2014; Zheng, Shi, et al., 2021).

3.3.2. Proteolysis and amino acid metabolism in cheese

Protein hydrolysis is a crucial biochemical reaction in cheese flavor formation, influencing the release and taste of flavor during the ripening process (Christen & Reineccius, 1995; Gan et al., 2016). Milk proteins, such as caseins, are decomposed from protein by protease in cheese, leading to the formation of flavor substances. Free amino acids, produced through proteinases and peptidases, are precursors to flavor compounds during cheese production and ripening. Free amino acid content and metabolism in mature cheese play essential roles in flavor development(Fox et al., 2015; Suzuki-Iwashima et al., 2020). Enzymatic removal of amino terminus leads to the formation of flavor and aromatic substances, such as 3-methyl-butanol, methionyl-propyl aldehyde, sulfides, and aromatic esters (Christen & Reineccius, 1995; Fagan et al., 2017; McSweeney et al., 2017; Tekin & Hayaloglu, 2023)

Branched-chain amino acids (Leu, Ile and Val), which are the precursors of aromatic compounds, are found in different cheeses. Isoleucine, leucine, and valine can be decarboxylated into isobutyl ester, 2-methylacetaldehyde, and ketoisocaproate, all of which have strong unpleasant aromas(Fox et al., 2015; O'Brien & O'Connor, 2017; Suzuki-Iwashima et al., 2020). Aromatic amino acid catabolism begins with a transamination step, producing compounds like α-keto acids, ammonia, and amines, which are further transformed into alcohols, esters, and acids. Ammonia is also an important flavor substance in many cheeses, such as Camembert and Gruyère. In cheese, methionine is converted to volatile sulfur compounds, such as methanethiol and dimethyl sulfide and dimethyl trisulfide, which represent the basic flavor substances in many cheese varieties. S-

Compounds contribute to the characteristic aroma of cheddar and the garlicky smell of well-ripened Camembert (Fox et al., 2015; Tekin & Hayaloglu, 2023; Zheng, Shi, et al., 2021). Proteolysis followed by oxidative decarboxylation of amino acids may produce low-molecular-weight biogenic amines (BA), which can cause adverse physiological reactions (Spano et al., 2010; Wójcik et al., 2021).

3.3.3. Biogenic amines in cheese

Biogenic amines (BAs) represent a group of low-molecular-weight organic bases produced as natural byproducts of metabolic processes in various biological entities, including humans, animals, plants, and microorganisms (Figure 1). In cheese production and consumption, BAs have garnered considerable attention due to their potential implications for food safety and human health. The main BAs detected in cheeses are histamine, tyramine, putrescine, cadaverine, and 2-phenylethylamine (Linares et al., 2011). The presence and concentration of these BAs in cheese are influenced by a multitude of factors, including the type of cheese, methods of seasoning, and the microbial composition of the cheese's microflora. Recent studies showed the dynamic nature of BA concentrations in different cheese varieties, revealing significant variations across different types and stages of cheese maturation (Barone et al., 2018; Zdolec et al., 2022). In semi-hard cheeses, for instance, tyramine concentrations have been reported to reach levels up to 1029 mg/kg at expiry, showing the potential for significant BA accumulation over time. Conversely, hard cheeses have demonstrated more stable and consistently high levels of tyramine and histamine, hovering around 1025 mg/kg during storage periods. Blue cheeses have exhibited a predilection for tyramine and cadaverine, with concentrations peaking at approximately 1306 mg/kg (Zdolec et al., 2022). The significance of microbial impact on the production of BA in cheese should not be underestimated. BAs are a class of naturally occurring toxic substances that are produced through microbial-mediated mechanisms, primarily involving the metabolism of amino acids and proteolytic activities. Hence, the microbial condition of cheese, including the composition and activity of its microflora, plays a pivotal role in determining the BA content of the final products (Barone et al., 2018; Ma et al., 2020).

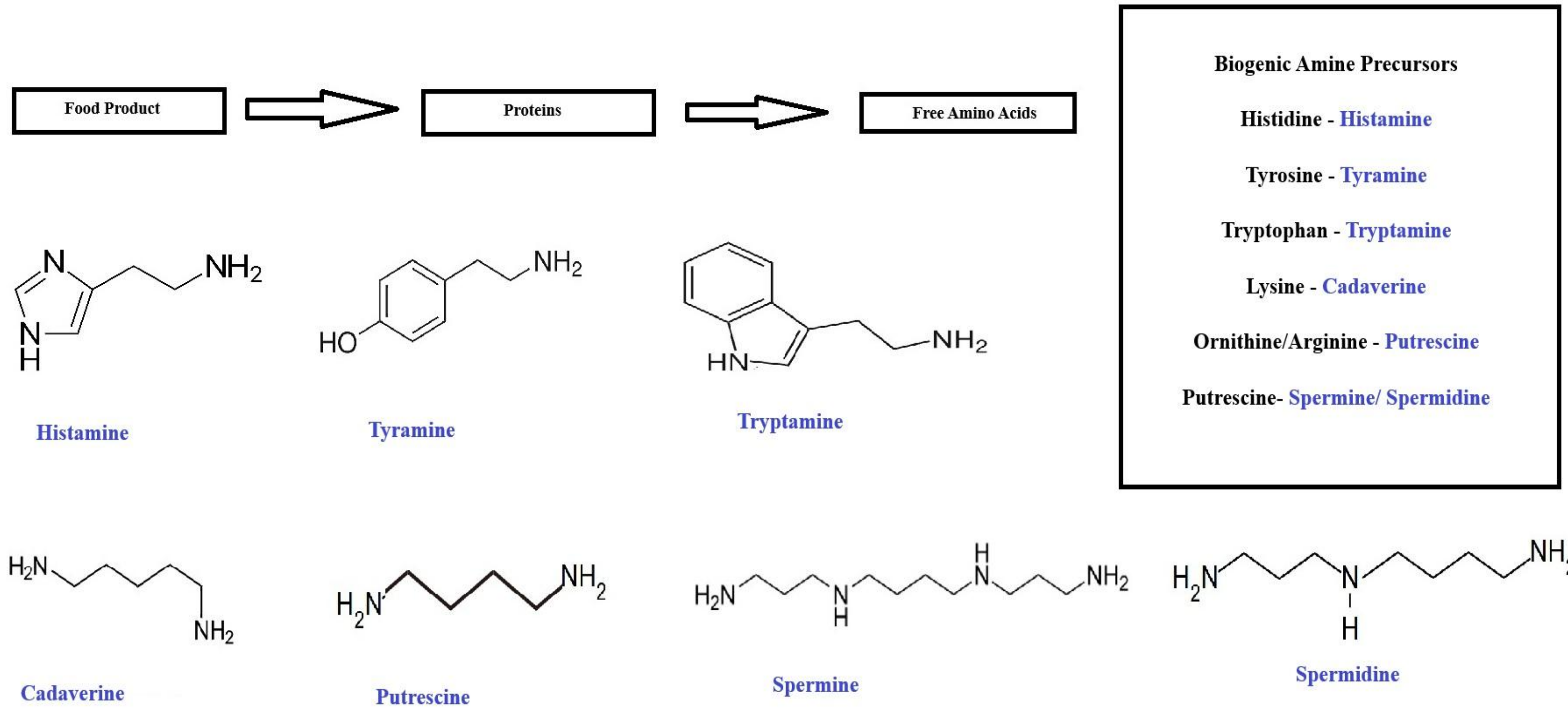

Figure 1. Structure of main biogenic amine detected in food

3.3.4. Lipolysis and fatty acid metabolisms

Cheese owes its multifaceted flavor and distinctive texture to intricate biochemical processes, with lipolysis playing a pivotal role(Caputo et al., 2021). Derived from various sources such as milk, rennet, starter cultures, auxiliary starters, nonstarter bacteria, and exogenous enzymes, lipases catalyze the hydrolysis of milk fat triglycerides (Caputo et al., 2021; Collins et al., 2003; Hickey et al., n.d.; McSweeney et al., 2004). Lipoprotein lipase (LPL) significantly influences flavor development in raw milk cheeses, while its impact on pasteurized milk cheeses remains minimal. Within lactic acid bacteria (LAB), lipolytic enzymes transform substrates into free fatty acid esters, triacylglycerides, diacylglycerides, and monoacylglycerides. Additionally, phospholipase A2 (PAB) contributes to characteristic flavor development through carbon dioxide production and the formation of free acids. Fatty acids, particularly free fatty acids like acetic, octanoic, and decanoic acids, emerge as essential contributors to cheese flavor(Caputo et al., 2021; Collins et al., 2003). The continuous volatilization of water during ripening shapes the unique texture and firmness of cheeses. Furthermore, γ- and δ-lactones, featuring five- and six-membered rings, impart intense aromas, while esters formed during milk fat degradation create sweet, fruity, and floral notes (Caputo et al., 2021; Collins et al., 2003; Rodríguez-Pinilla et al., 2015).

3.4. Meat

3.4.1. Meat and meat composition

Meat is a crucial, nutritious, and energy-rich natural food product used by humans for maintaining a healthy diet and achieving optimal human growth and development. It is derived from domestic animals, poultry, farmed, and wild animals and is rich in high-value proteins, fats, vitamins, and minerals (Pereira & Vicente, 2013; Wood, 2017). Most of the world's population regularly consumes animal protein, including pig meat, cattle meat, lamb meat, goat meat, poultry, and other types of meat. Among these, cattle meat, commonly known as beef, stands out due to its distinct taste and nutrient content. Worldwide beef meat production is estimated to reach approximately 60 million tons annually, with the United States, Brazil, China, and Argentina leading as the largest producers (World Food and Agriculture – Statistical Yearbook, 2023)

Meat composition varies based on breed, feed type, climatic conditions, and meat cut, affecting its nutritional and sensorial properties (Table 3). Meat is a rich source of essential amino acids, minerals, essential fatty acids, and vitamins, with organ meat like liver being an enriched source of Vitamin A, Vitamin B1, and nicotinic acid (Wood, 2017). Research is ongoing to understand the differences in nutritional value between different meat cuts, animal species, and breeds. Meat with less connective tissues is likely to have lower digestion and absorption scores, while meat with more connective tissues has less essential amino acid content, making it less nutritious. However, meat with more connective tissues has more digestibility and nutritional value (De Smet & Vossen, 2016; Wood, 2017).

Water is a crucial component of all food materials, with three types: perishable commodities (having more than 70% moisture content), non-perishable commodities (having around 50-60% moisture contents), and stable food materials (with less than 15% moisture). Meat ranks among the perishable food material, as it contains around 70% of moisture. Meat tissues contain a major portion of water within muscle fibers, while smaller amounts are present in connective tissues. Processing conditions like curing and heat treatment can alter the water holding ability of meat, enhancing its shelf life (Ahmad et al., 2018; Kamruzzaman et al., 2016; Wood, 2017).

Carbohydrates in the animal body are stored in the form of "glycogen" mainly in the liver and muscles. The conversion of stored glycogen to glucose and from glucose to lactic acid is governed by hormones and enzymes(R. S. Ahmad et al., 2018; Jensen et al., 2011). During the early stage of aging, the lactic acid content of muscles increases, lowering the pH. If an animal suffers from severe stress or exercise just before slaughter, a minute amount of glycogen will convert into lactic acid, causing an elevated pH (6.5) and dark, firm, and dry (DFD) condition. Fast postmortem causes a drop in muscle pH (5.0) and is recognized by pale, soft, and exudative condition (PSE), which is common in pork meat (Adzitey & Nurul, 2011). The nutritional value of meat is significantly influenced by the carbohydrate contents of meat.

Proteins naturally occurring complex nitrogenous compounds usually found in high amounts in meat and meat products. Meat has a high score of protein digestibility corrected amino acid score (PDCCAS) 0.92 compared to other protein sources, such as lentils, pinto beans, peas, and chickpeas. PDCAAS is based on an age-related amino acid reference pattern and estimates of the protein's digestibility. The score is assumed to predict biological value, or the anticipated ability of the absorbed test protein to fulfill human amino acid requirements. Protein utilization is predicted from expected digestibility and the amino acid composition of the protein (Millward, 2012). Meat provides between 20% and 40% of protein in the diet, with the amino acids and their proportions like those in human muscles (Corbin et al., 2015; Wyness et al., 2011). Meat contains eight essential amino acids, which cannot be synthesized in the body, and contains all of these. In a study of beef and sheep produced in New Zealand, lean meat provided 80%-110% of the recommended daily intake of most amino acids but a lower percentage of branched chain amino acids (isoleucine, leucine, and valine)(Purchas et al., 2014).

Fats are one of the three major macro-nutrients, along with carbohydrates and proteins. They are known as triglycerides, esters of three fatty acid chains and alcohol glycerol. Meat contains fatty tissues that have varying amounts of fat, serving as energy deposits, protective padding in the skin and around organs, especially the heart and kidney, and providing insulation against body temperature losses (Aranceta & Pérez-Rodrigo, 2012; Enser et al., 1998; Wyness et al., 2011). Fat content in animal carcass varies from 8 to 20%, with the latter being only found in pork (Enser et al., 1996, 1998). Lean from red meat contains more fat than that from poultry, and for meat to be labeled as low-fat food, fat content should be less than 3 g/100 g according to EC regulations (EC, 2006).

Table 3. Macronutrients in different types of meat (per 100g)

	Beef	**Sheep**	**Pork**	**Chicken**	**Turkey**
Water (g)	71.90	70.60	74.00	75.10	75.30
Protein (g)	22.50	20.20	21.8	22.30	22.60
Fat (g)	4.30	8.00	4.00	2.10	1.60

*Source: Adapted and modified from (Wood, 2017).

Minerals are essential nutrients in food materials for the human body's growth, development, and maintenance. They are divided into macro- and micro-minerals based on their requirements. Macro-minerals include sodium, calcium, phosphorus, magnesium, chloride potassium, sulfur, and iron, while micro-minerals include iron, zinc, iodine, copper, cobalt, manganese, selenium, and fluoride (Soetan et al., 2010). Raw meats are not important sources of sodium in the diet, but meat and meat products contribute significantly to sodium intake. In the

UK, 26% of sodium intake is obtained from meat and meat products, as salt is widely used in the food industry as a preservative and flavor enhancer(Henderson et al., 2003). Meat is either a "source" or a "rich source" of phosphorus, contributing 23% to the UK diet. Beef is a "source" of iron in diet, and liver is a "rich source." Iron is present as heme, with higher bioavailability than nonheme iron. Copper is present at low levels in lean meat but is very high in the beef liver(Henderson et al., 2003; Wood, 2017). Zinc is an integral part of key enzymes in the body, and meat in the diet enhances the absorption of zinc from other foods. Selenium is present at useful levels in lean meat and contributes significantly to the diet, with meat contributing 21% in Denmark (Rooke et al., 2010).

Vitamins are essential organic substances in the human body, crucial for growth, development, and maintenance. They are essential for children and are not prepared by mammalian cells and must be supplied through diet. Vitamins are classified into water- and fat-soluble vitamins, with water soluble vitamins including B-complex vitamins and vitamin C, and fat-soluble vitamins of meat including vitamin A, vitamin D, and vitamin K(Purchas et al., 2014; Soetan et al., 2010; Wyness et al., 2011). Meat and meat products are the only natural sources of vitamin B12, which is required for neurotransmitter and DNA synthesis and enzymes involved in fatty acid and amino acid metabolism. Meat provides more than a third of the daily intake of vitamin B3 (niacin)(De Smet & Vossen, 2016; Henderson et al., 2003).

Other vitamins, such as A, C, D, and E, are not present in high amounts in lean meat, although vitamin A concentrations are high in liver. Vitamin D, produced by activation of 7-dehydrocholesterol in the skin by sunlight, is important for people who do not expose themselves to ultraviolet light. Vitamin E is an important antioxidant in the body, and supplementation with high levels of vitamin E is now common practice in many countries (Danish Institute for Food and Veterinary Research, 2005; Rooke et al., 2010; Soetan et al., 2010).

3.4.2. Proteins and amino acid composition of meat

Meat ranks among one of the protein-rich foods, providing high biological value to the masses. Meat muscle contains 19% proteins (11.5% structural proteins, 5.5% soluble sarcoplasmic proteins, and 2% connective tissue)(Bender, 1992) . The protein content is modified in cooked meat due to water loss through the cooking process, making them highly digestible (94%)(Williams, 2007).

Protein quality is crucial in understanding the nutritional value of a food as a protein source. The Digestible Indispensable Amino Acid Score (DIAAS) is used to calculate protein quality, which is based on the content and digestibility of indispensable amino acids (IAA) in a food. Meat has a DIAAS value of 0.8-1.4, while traditional plant proteins have lower scores. Meat is an excellent complement to plant-sourced proteins, as its high DIAAS value makes it an excellent source of protein (Marinangeli & House, 2017). Meat being rich in protein is a great source of amino acids which plays important roles in human nutrition and health. Amino acids and bioactive compounds play a crucial role in human health, including preventing muscle-wasting diseases like sarcopenia, reducing calorie intake, controlling blood pressure, and maintaining intestinal functionality(McNeill, 2014; Phillips, 2012; Young et al., 2013). Amino acids like leucine, isoleucine, and valine are essential for protein synthesis, with leucine supplementation increasing muscle protein synthesis in older adults(Wall et al., 2013). Protein ingestion also increases muscle protein synthesis rates due to the stimulatory effect of essential amino acids. Beef, rich in glutamic acid/glutamine, arginine, alanine, and aspartic acid, has been found to stimulate muscle protein synthesis in an insulin-dependent and independent manner. Beef is rich in branched-chain amino acids, leading to further metabolic effects(Bender, 1992; Wall et al., 2013; Williams, 2007). Meat proteins provide all essential amino acids, including lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, and valine, with no limiting amino acid.

3.4.3. Biogenic amine composition of meat

Meat and meat products are particularly subjected to biogenic amine production due to their high protein and amino acid content, and the proteolytic activity can arise because of prolonged storage or in association with the production process (Schirone et al., 2022). Factors associated with raw materials, such as pH and chemical composition, handling and manufacturing operations, temperature, and time of storage, may influence their presence in foods (Ekici & Omer, 2020; Papageorgiou et al., 2018; Stadnik & J.Dolatowski, 2010). Moreover decarboxylase-positive microorganisms (e.g. *Enterococcus, Lactobacillus, Carnobacterium, Pediococcus, Lactococcus,* *and Leuconostoc*) can be present in raw materials and/or introduced by contamination before, during, or after processing which leads in BA formation (Ekici & Omer, 2020).

Bacterial amines (BAs) are found in meat and various meat products, including fermented and cured meat products. Fermented meat products are a primary source of BAs due to their manufacturing process, which promotes bacterial and fungal activities. Microorganisms take advantage of available nutrients, favorable water activity, and anaerobic conditions in these products. The presence of pH, salt, and low-containing sugar environments can also diminish BAs formation by microorganisms(Jairath et al., 2015; Schirone et al., 2022; Stadnik & J.Dolatowski, 2010). Fermented products like salamis and sausages can pose health hazards for the accumulation of tyramine (Tym), histidine (Him), putrescine (Put), and cadaverine (Cad). Tym and His are linked with direct side effects on the human body, but the presence of other amines can even boost their activity (Durak-Dados et al., 2020; Jairath et al., 2015; Stadnik & Dolatowski, 2010).

Biogenic amines, found in meat and meat products, are potential precursors of stable carcinogenic N-nitrosamines and can enhance the growth of chemically induced aberrant crypt foci in the intestine. The formation of N-nitroso compounds is an additional toxicological risk associated with biogenic amines, especially in meat products that contain nitrite and nitrate salts as curing agents. Primary biogenic amines can convert to secondary amines during heating and storage at room temperature, leading to further reactions with nitrite (De Mey et al., 2014; Xie et al., 2023).

Biogenic amines are important for two main reasons: their toxicological effects due to intake of foods containing high concentrations of biogenic amines and their interaction with some medicaments, and their use as biogenic quality index roles as indicators of quality and/or acceptability in some foods (Jairath et al., 2015). The severity of the clinical symptoms caused by BAs depends on the amount and variety ingested, individual susceptibility, and the level of detoxification activity in the gut (Wójcik et al., 2021). Biogenic amine quality indexes have been used to assess meat quality and changes in biogenic amine content in different meat derivatives(Bardócz, 1995; Kalač, 2014; Wójcik et al., 2021). However, applying similar quality criteria to fermented products has proven more difficult due to the number of factors involved in their formation.

Legal limits for biogenic amines in food products are difficult to determine due to their effect not being dependent on their presence alone but also influenced by other compounds and the specific efficiency of detoxifying mechanisms in different individuals. Histamine is the most studied amine about its toxicological effects, with various limits set by the European Union (EU) and the United States Food and Drug Administration (USFDA). The EU has established regulations for histamine levels in raw fish, salted fish, and cured products with a maximum of 100mg /kg , 200 mg/kg and 400 mg/ kg, respectively (EFSA, 2011), while the FDA has established a 50 mg/kg for histamine in seafood (FDA, 2002).

3.4.4. Fat and fatty acids in meat

Fatty acids in meat are found in adipose tissue, cell membranes, and muscle. They are contained in triacylglycerol and phospholipids, which form a major part of all cell membranes. Fatty acids are classified into three types: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)(Woods & Fearon, 2009). The total fatty acid composition of muscle is significantly different between samples with low total fat and high total fat (Raes et al., 2001; Scollan et al., 2014).

The fatty acid composition of beef, sheep, pork, and chicken is influenced by production factors, particularly the feed regime (Nogoy et al., 2022; Woods & Fearon, 2009). Muscles from ruminant species have higher percentages of saturated fatty acids (SFA) and lower percentages of polyunsaturated fatty acids (PUFA) than monogastric species, such as pork and chicken. This difference is due to the breakdown of dietary PUFA to SFA and MUFA in the rumen by bacterial biohydrogenation (Enser et al., 1998; Raes et al., 2001; Woods & Fearon, 2009).

Meat contains essential unsaturated fatty acids, including oleic, linoleic, linolenic, and arachidonic acids, which are essential for mitochondria and metabolic sites. Linoleic acid is abundant in vegetable oils, while eicosapentaenoic and docosahexaenoic acids are present in fish and fish oils. The main dietary PUFA, Linoleic acid (C18:2 n-6), is essential for metabolism and cannot be synthesized in the body. The other essential fatty acid, 18:3 n-3 (a-linolenic acid, ALA), is also present in plant oils but at lower levels than LA. The presence of ALA in the muscles of beef and sheep leads to the formation of metabolically important long-chain n-3 PUFA, including 20:5 n-3 (eicosapentaenoic acid) and 22:6 n-3 (docosahexaenoic acid)(Pighin et al., n.d.; Scollan et al., 2014; Wood et al., 2004). Fatty acids are also involved in several physicochemical properties of meat, contributing not only to the nutritional attributes of meat but also to the physicochemical ones. Marbling fat, total fatty acid content in muscle, has been long recognized as a quality factor of meat and positively associated with juiciness and tenderness (Corbin et al., 2015; Dilzer & Park, 2012b; P. M. de C. C. Pereira & Vicente, 2013; Wood, 2017; Woods & Fearon, 2009).

3.4.5. Meat products composition

Meat products are a cornerstone of many diets worldwide, providing essential nutrients necessary for human health. High-quality proteins, which contain all the essential amino acids, are one of the most critical components of meat. Biesalski, (2005) emphasizes that meat proteins are easily digestible and crucial for muscle development and maintenance. Additionally, meat is a rich source of vital micronutrients such as iron, zinc, and vitamin B12. The bioavailability of heme iron in meat is significantly higher than that of non-heme iron found in plant sources, making it an important food for preventing iron deficiency anemia (Hurrell & Egli, 2010). However, the fat content in meat varies widely among different types of meat products. Lean meats, such as poultry and certain cuts of pork and beef, contain less saturated fat compared to processed meats like sausages and bacon. Micha et al. (2010) found that high consumption of processed meats is associated with an increased risk of cardiovascular diseases due to their high saturated fat and sodium content. Thus, choosing leaner cuts and moderating the intake of processed meats can contribute to a healthier diet.

The processing of meat products encompasses a range of traditional and modern techniques aimed at improving shelf life, safety, and sensory qualities. Traditional methods such as smoking, curing, and fermenting have been used for centuries. While these methods enhance flavor and preserve meat, they can also introduce potentially harmful compounds like nitrosamines and polycyclic aromatic hydrocarbons (Lorenzo et al., 2018; Ribeiro et al., 2019). Modern processing technologies have been developed to address some of these concerns. High-pressure processing (HPP) is one such innovation that inactivates pathogens and extends shelf life without significantly affecting the nutritional value of the meat (Tao et al., 2014). Additionally, sous-vide cooking, which involves vacuum-sealing meat and cooking it at precise low temperatures, has gained popularity for its ability to maintain meat quality and improve tenderness. Despite the benefits of these technologies, the impact on the sensory qualities of meat, such as texture, flavor, and color, remains a critical consideration. Consumers often prefer the unique textures and flavors achieved through specific processing techniques, highlighting the importance of balancing safety and quality in meat processing (Toldora, 2010).

Meat products such as sausage, dry meat and ham are widely consumed due to their unique flavors, convenience, and nutritional value. These products undergo various processing techniques which influence their sensory characteristics, shelf-life, and safety. Sausages dry meat and ham are made from different types of meat, often pork, beef, or poultry, combined with fat, salt, and various additives. The quality and type of meat significantly influence the final product. According to a study by Zhang et al., (2013), the choice of meat, fat content, and the addition of spices and herbs play crucial roles in defining the flavor profile and texture of sausages.

The processing techniques for sausages typically include grinding, mixing, stuffing, and cooking or curing. Dry meat and ham production involves curing, which can be dry or wet, followed by smoking and aging. These processes contribute to the development of characteristic flavors and extend the shelf-life of the products. For instance, Toldrá et al. (2012) emphasize that the curing process in ham enhances its sensory attributes by promoting proteolysis and lipolysis, which generate flavor compounds.

Sausages dry meat and ham are rich in protein, essential amino acids, vitamins, and minerals. However, their nutritional profile can be influenced by the presence of fat, sodium, and additives. A study by Arihara (2006) highlights that while these products are excellent protein sources, they can also be high in saturated fats and sodium, which are linked to various health issues when consumed in excess. Efforts to improve the nutritional profile of these meat products have led to the development of low-fat and low-sodium alternatives. For example, the use of plant-based ingredients and leaner cuts of meat are strategies employed to reduce fat content. Additionally, potassium chloride and other salt substitutes have been investigated to lower sodium levels without compromising taste.

4. MATERIAL AND METHODS

4.1. Material

4.1.1. Busha milk samples

Milk samples: Samples were collected in the Dukagjin area of Kosovo (Stellc in the Peja region; Rrenc and Xerc in the Prizren region), where this breed is more prevalent than in other areas. Twenty 100 ml milk samples were collected from two Busha cattle strains, Sharri and Dukagjini. Conventional sampling techniques were used to ensure the collection of representative samples for subsequent analysis, following the guidelines outlined in CODEX STAN 234-1999. All milk samples were collected from cows at a comparable mid-lactation stage (approximately 2–4 months postpartum) to minimize physiological variability.

4.1.2. Kosova Traditional homemade cheese samples

Soft cheeses: Soft cheeses produced in the traditional way were provided by three local cheese producers of Kosovo. These cheeses were produced using self-fermentation for curd production as explained by Bytyqi et al. (2017) from the unpasteurized milk of local cows, goats, and buffaloes. Briefly, 8-10 kg of unpasteurized milk was left to self-ferment in a container for two days. After this period, the cream layer on the surface was removed, and the curd was heated to 40 °C for 30 minutes. The curd was then cut to release the whey and left to dry at room temperature for 12 hours. Finally, the curd was pressed with a heavy pot and salted in brine (Figure 2). Three parallel samples, each 500 g, were prepared.

Sharri Cheese: Three parallel cheese samples of 500 g were provided from the Prizren region, more specifically from the Sharri mountains in Kosovo. The technological process of preparation of Sharri cheese for this study was as described by Bytyqi et al., (2017). Firstly, sheep milk was thermally treated up to 30°C for 30 minutes, followed by a coagulation process using Valiren rennet (4g/100 l). The syneresis took place for 35 minutes, with curd processing taking 2-5 minutes, stirring lasting for 15 minutes, and sedimentation for 10-15 minutes. After the whey were removed, the cheese curd underwent thermal processing using hot water at a temperature of 90°C. Sharri cheese was drained for 12 hours within a cheesecloth, giving the "bread" shape of Sharri cheese. Sharri "bread cheese" was salted and set to pre-ripe for 14 days on wooden boards at a temperature of 10-12 °C, with a relative humidity of approximately 70-75%. After the preripening process, the bread cheese was hand-broken into small pieces (100-150 g) and set to the final repining stage for 30 days (10-12 °C; relative humidity of approximately 70-75%). Sharri cheese was transferred in brine with a concentration of 12%, and parsley was added to increase the taste and aroma of the cheese (Figure 3).

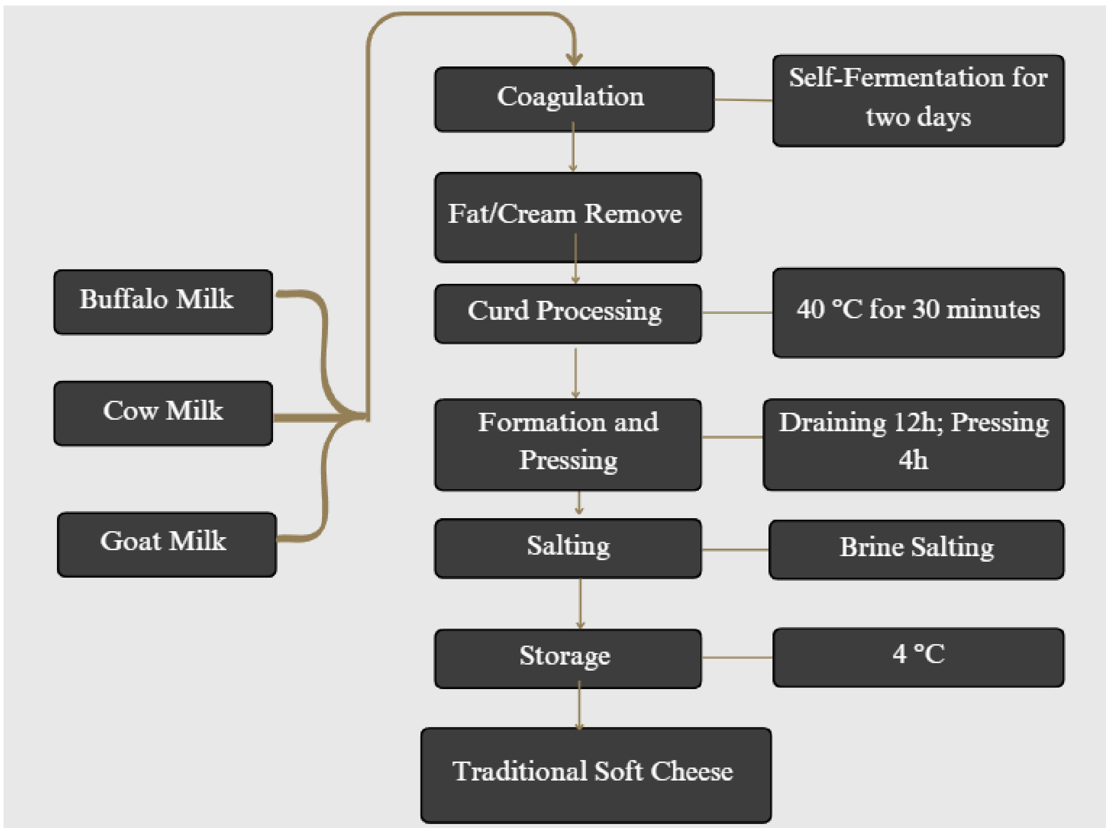

Figure 2. Technological process of soft buffalo, cow and goat cheese

Rugova cheese: Rugova cheese sample was provided in Peja region, specifically in Rugova in Kosovo. Rugova cheese was made of local breed cow milk. After fresh milk was heated up to 30°C, it was clotted with powder enzyme (about 1.5 g enzyme and 7 g salt) for one hour. Then the following process was cutting curd and draining for 12 hours to remove the whey. After the whey was removed, the cheese was pressed (pressing with heavy weights) for one hour and then salted. Firstly, dry salted for 24 hours, and then moved to bring. The cheese was ripened for 60 days in brine at 5°C. Three parallel samples were taken for analysis (Figure 4).

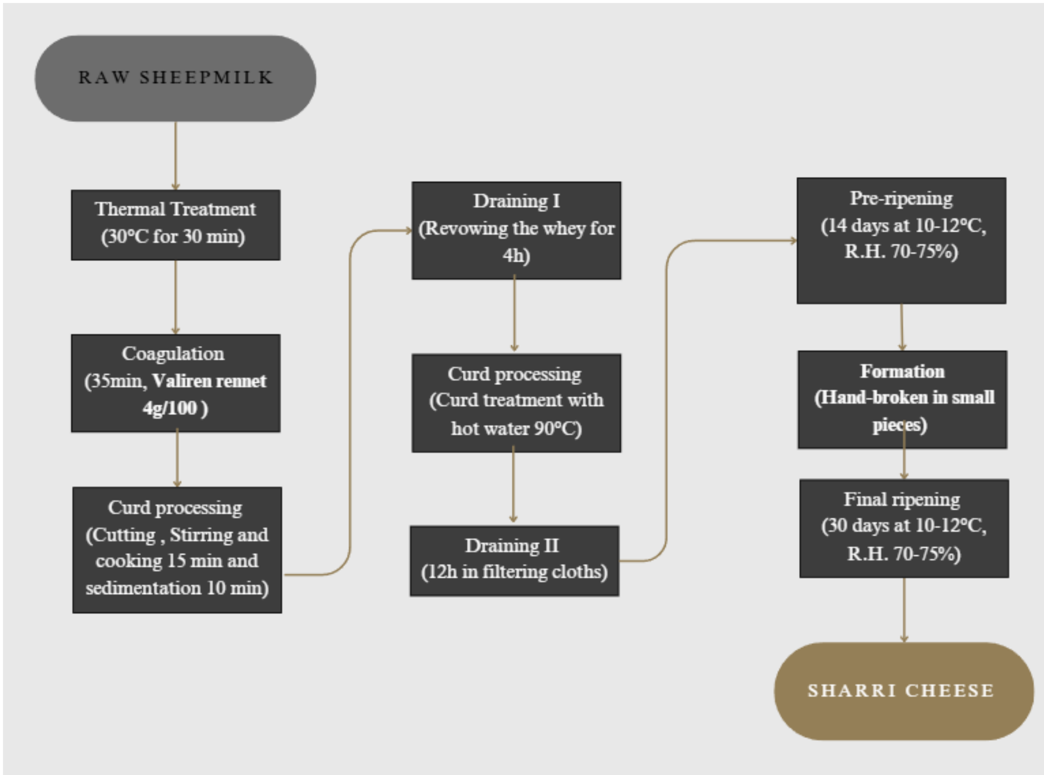

Figure 3. Technological process of Sharri cheese preparation

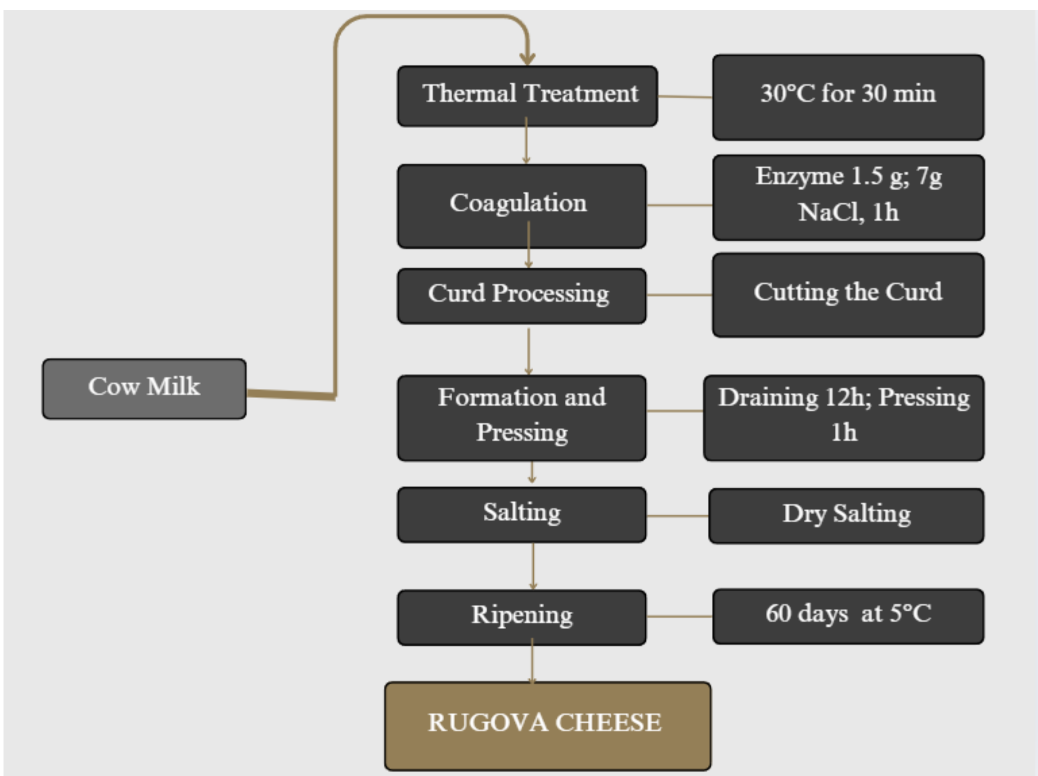

Figure 4. Technological process of Rugova Cheese

4.1.3. Traditional Beef meat products samples

Traditional dry beef meat: Three parallel samples of dried beef meat were taken for the analysis**.** The beef drying process entails utilizing meat obtained from mature, low-fat animals. The meat was meticulously separated from the skeletal structure following the removal of the fatty tissue and any connecting structures. The meat was cut precisely so that uniformly thick, long strips are produced. The cutting process followed the natural orientation of muscle fibres. The lengths of the resultant strips range from 20 to 70 cm, and their cross-sectional area measures 1 cm x 1 cm. Alternatively, they can be thinly sliced sections with dimensions of 0.5 cm × approximately 4 cm. The dry salting method involved the even distribution of salt after each layer of meat, constituting approximately 14% of the total weight. The cured meat was left undisturbed for a duration of 12 hours prior to commencing the drying process. The subsequent drying process involved drying for a period of 4-5 days at a controlled temperature range of 50-60°C (Figure 5).

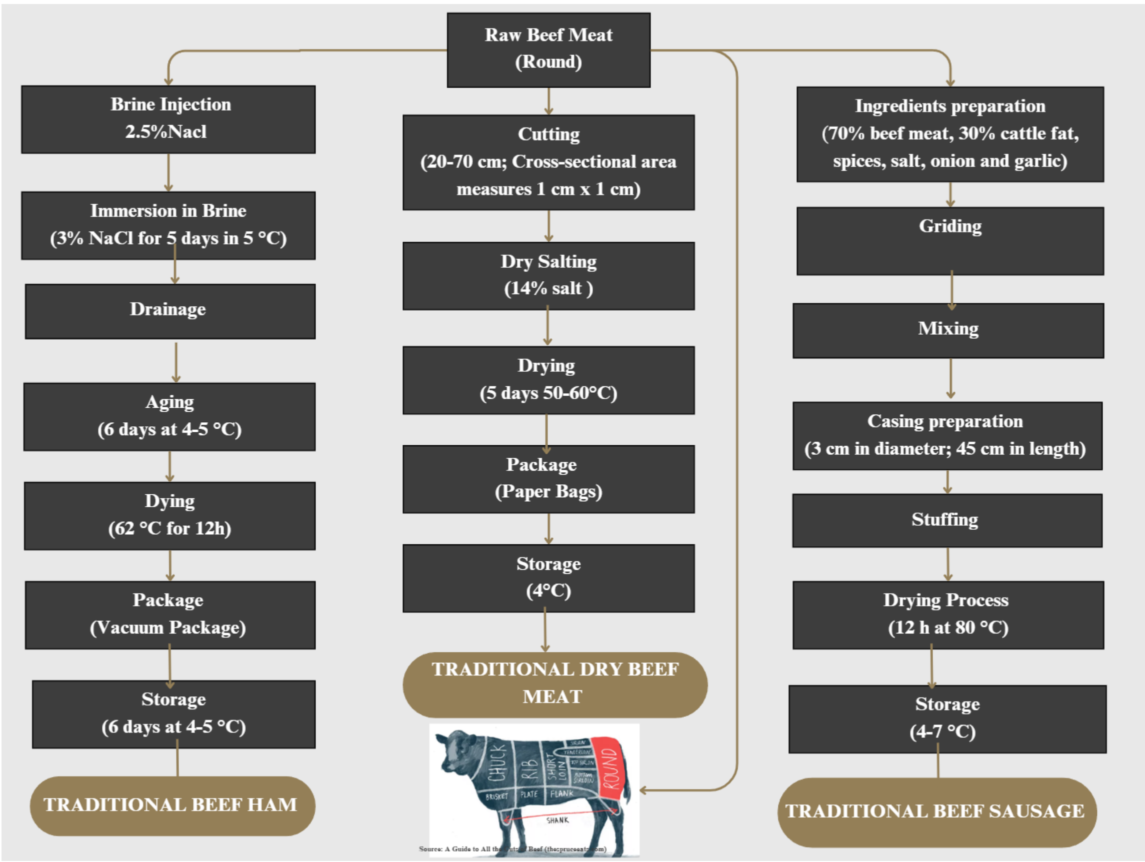

Figure 5. Technological process of preparation of traditional beef meat products

Beef Ham: Three distinct samples of beef ham were prepared. The beef ham samples were provided by a local Kosovar manufacturer and were produced using the following protocol: Initial processing involved the segmentation of round beef meat into small geometric configurations, followed by the injection of brine (2.5% NaCl). Subsequently, immersion in a brine solution containing 3% NaCl was conducted for a duration of 5 days, maintaining a temperature of 5°C. Post-immersion, the samples underwent a draining phase, succeeded by an aging period spanning 6 days within a controlled temperature range of 4-5°C. The ultimate stage of the preparation process involved subjecting the beef ham samples to a drying procedure at 62°C for a duration of 12 hours (Figure 5).

Beef Sausage samples: For this study, three samples of homemade beef sausage were prepared. The sausage was provided by a local sausage manufacturer in Kosovo, produced by a recipe that included 70% beef meat, 30% fat (from cattle), as well as spices, salt, onion, and garlic. The ingredients were ground using an Alexanderwerk cutter (ALEXANDERWERK, Germany) and then filled into round beef casings that were 3 cm in diameter and 45 cm in length (34/36 AB 15m"). The sausage was dried for 12 hours at 80 °C in an air master chamber. This type of sausage is raw sausage do not undergo the fermentation process and has a shelf life of 3 months when stored in a fridge at temperatures between 4-7 °C. However, it can be stored for up to 1 year if frozen. The analysis of the homemade beef sausage samples was conducted immediately after production (Figure 5).

4.2. Moisture content and protein determination

The ISO1442:1973 method was used to determine the moisture content. The protein content was determined based on the Kjeldahl method (Kjeldahl, 1883).

4.3. Amino acid determination

4.3.1. Protein building amino acids

The composition of protein-building amino acids in milk. cheese and beef meat products was determined after HCl hydrolysis method described by Berisha et al. (2023). Samples were analyzed using an Automatic Amino Acid Analyser AAA400 (Ingos Ltd., Prague, Czech Republic) fitted with an Ionex Ostion LCP5020 cation-exchange column (22 x 0.37 cm).

4.3.2. Free amino acids

For free amino acids (FAA) determination 3 g of traditional cheeses and beef meat products was extracted with 10 ml 10% trichloroacetic acid for one hour at room temperature at 100 rpm using a Laborshake as described by Berisha et al. (2023). The same equipment was used as for the protein building amino acids determination.

4.3.3. Amino acid Score calculation

The World Health Organization calculation method, as given in equation 1, was followed in this study to determine the essential amino acid scores, also referred to as chemical scores. The scores were presented as either a unity ratio, as suggested, or as a percentage scale, following the guidelines from the (Food and Agriculture Organization Expert Working Group, 2018)

The reference protein quantities were divided into three age groups: 3-14 years, 15-18 years, and individuals over 18 years, as authorized by the (Food and Agriculture Organization Expert Working Group, 2018)

$$Amino\ Acid\ Score = \frac{\text{mg of amino acid in 1 g of the tested protein}}{\text{mg of amino acid in 1 g of the reference protein}} \qquad eq\ (1),$$

4.3.4. Essential amino acid deficiency index calculation

The essential amino acid deficiency index was calculated by dividing the essential amino acid content of the sample with that of the reference protein. If the disparity is positive, it means there is a lower proportion of the amino acid in the sample compared to the reference protein. A negative value indicates an excess of the amino acid. Therefore, positive values indicate a shortage. The essential amino acid deficiency index is calculated by adding up these positive differences.

4.4. Biogenic amine determination

The sample preparation for biogenic analysis is the same as explained above for free amino acids. Biogenic amines were determined with the same equipment using different ion-exchange column (Ostion LG ANB ion-exchange resin; 7.0×0.37 cm). Separation was carried out using

Na+/K+ buffers system by stepwise gradient elution. Detection was performed at 570 nm (Berisha, et al., (2023a) Berisha, et al., (2023b)

4.5. Fatty acid determination

Sample preparation followed the Bligh and Dyer (1959) method, where samples were extracted using petroleum ether via a Soxhlet extractor.

The fatty acid ester preparation and the Gas chromatography determination was done according to (Berisha, et al., (2023a) for cheese samples and Berisha, et al., (2023b) for beef meat products.

4.6. Statistical analysis

The statistical analyses for this study were performed using IBMSPSS25 software (Peck et al., 2020). For Busha milk, significant differences between the means for conductivity were evaluated using a one-way analysis of variance (ANOVA) using Tukey's test with a significance level at $p < 0.05$. On the other hand, significant differences between the means for protein, lactose, fat, total solids, amino acids, and biogenic amines were determined by a multivariate analysis of variance (MANOVA), using Tukey's test at $p < 0.05$. The normality of the differences was accepted using the Shapiro-Wilk and D'Agostino tests, while Levene's test confirmed the homogeneity of variances.

The quality parameters were compared using MANOVA at a significance level of $p < 0.05$ for the traditional cheeses and traditional beef meat products. The normality of the differences was tested and accepted using the Shapiro-Wilk and D'Agostino tests. Additionally, Principal Component Analysis (PCA) was performed to categorize the traditional cheese and beef meat product samples based on their protein-building amino acids, free amino acids, and fatty acids composition. PCA was performed using XLSTAT Software (Addinsoft, 2022).

5. RESULTS AND DISCUSSIONS

5.1. Busha milk composition

5.1.1. Dry matter content, pH, and conductivity of Busha milk

The average dry matter content in 2 Busha cattle breeds (Dukagjini, Sharri), which includes protein, fat, and lactose, ranged from 13.09% to 13.86% (Figure 6). Between the two cattle strains, the protein content was similar around 3.70%. The concentration of fat and lactose in Dukagjini cattle milk was higher (4.50% and 4.44% respectively) compared to Sharri cattle milk (4.18% and 4.2% respectively). The results of the MANOVA test indicated statistically significant differences ($P < 0.05$) in the total solid, fat, and lactose content between the milk samples of Sharri and Dukagjini cattle. Specifically, the Dukagjini Busha milk had higher values in these parameters. The milk's average pH value (pH 6.6) and conductivity (4.14 mS/cm) fell within the range for fresh milk, as defined by Caprita et al. (2014) for pH values between 6.5 and 6.7, and (Norberg et al., 2004) for conductivity values between 4.0 and 5.86 mS/cm. These findings suggest that the milk from Busha cattle is of high freshness and quality.

The amount of fat and protein of Busha cattle milk is as good as to other higher-productive breeds. According to a review paper by Samková et al. (2012), the fat and protein contents of various breeds' milks are as follows: the fat content of 2.0–6.1% and 2.5–3.8%, respectively, of Holstein cow milk. The fat content of brown Swiss cow milk ranges from 1.9% to 5.7%, while its protein content ranges from 2.4% to 4.1%. The fat content of Jersey cow milk ranges from 4.8% to 6.8%, while its protein content ranges from 3.7% to 4.6%. The fat content of Friesian cow milk ranges from 3.6% to 5.4%, while its protein content ranges from 3.1% to 4.1%. Czech Pied cow milk exhibits a fat content ranging from 2.7% to 6.0% and a protein content ranging from 2.8% to 4.4%. By comparing these compositions, the fat content of Busha cow milk (4.18–4.50%) generally falls within or slightly above the middle range of the listed breeds, and this range is consistent with the fat content observed across various breeds of cows. Busha cow milk has a slightly higher protein content than that of some breeds, such as Holstein and Czech Pied, but a lower protein content than that of other breeds, such as Jersey and Friesian. These differences in the fat and protein content of various breeds of cows also demonstrate the variety of milk's nutritional profiles, which may satisfy a range of dietary needs and preferences.

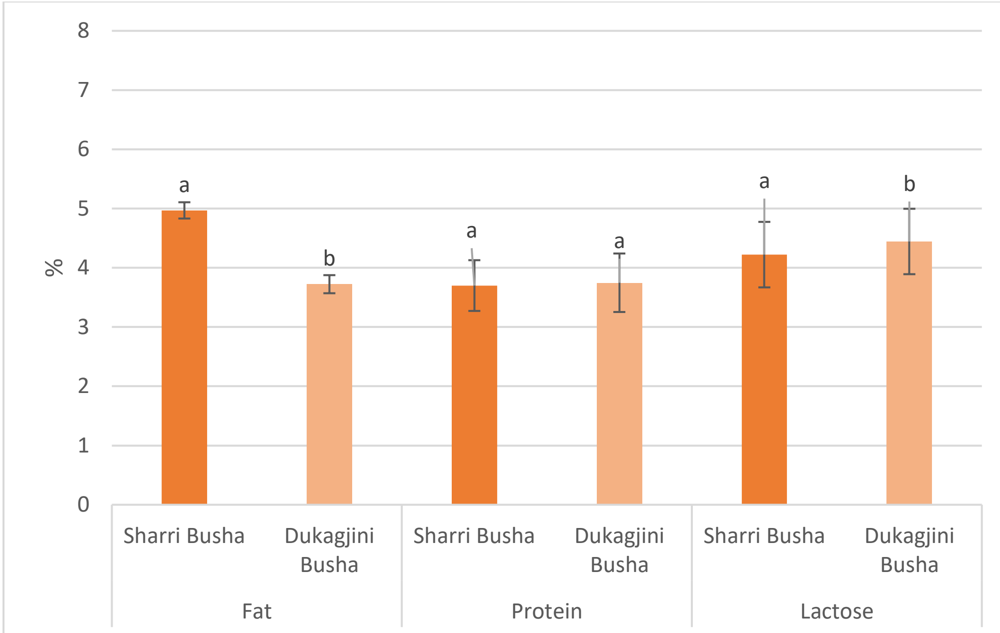

Figure 6. Fat, protein and lactose content of strains of Busha Cattle milk Number of sample n = 20; values are mean ± standard deviation; means with different lowercase superscripts differ (P < 0.05)

5.1.2. Protein-building amino acid composition of Busha milk

Mean values of protein-building amino acid compositions of the Busha cattle breeds milk is given in Table 4. The main amino acids were glutamic acid (24.54%), proline (14.04%), leucine (9.37), aspartic acid (6.50), lysine (6.97) and valine (5.50%), while the minor ones were glycine (1.67%) and cysteine (0–0.58%). The main amino acids represent around 68% of the total amino acid content of the Busha cattle milk. When comparing Busha milk with higher productive cow breeds several differences are seen. Busha milk has a higher percentage of Glu and Pro compared to other cattle breeds, including Holstein Friesian, Swedish, Czech Fleckvieh, and Holstein. The Glu content in Busha milk was 24.54%, which was significantly higher than in Swedish (Lindmark-Månsson et al., 2003), Czech Fleckvieh and Holstein (Křížová et al., 2013), Holstein Friesian and Brown Swiss (Bobe et al., 2009), which had 20.80%, 19.15%,19.15%, 17.19% and 15.19% Glu, respectively. Similarly, the Pro content in Busha milk was 14.04%, again higher than in Holstein Friesian (8.49%), Swedish (9.4%), Czech Fleckvieh (10.28%), Holstein (10.22%), and Brown Swiss (7.45%) (Bobe et al., 2009; Křížová et al., 2013; Lindmark-Månsson et al., 2003). On the other hand, the percentages of Leu, Lys, Asp, and Val in Busha milk were lower than in the other breeds. The Leu content in Busha milk was 9.73%, which was lower than in Brown Swiss (13.00%) Holstein Friesian (12.48%), Swedish (9.37%), Czech (9.47%), Fleckvieh (9.40%), and Holstein.

Table 4. Protein- building amino acid composition of milk Busha Breed

Protein building amino acids	mg/kg	%
Glutamic acid	880	24.54±0.10
Proline	423	14.04±0.23
Leucine	349	9.73±0.30
Lysine	250	6.97±0.18
Aspartic acid	233	6.50±1.8
Valine	197	5.50±0.46
Serine	188	5.24±2.30
Tyrosine	150	4.19±2.90
Phenylalanine	144	4.02±0.50
Threonine	138	3.85±0.30
Isoleucine	121	3.37±0.34
Arginine	105	2.93±0.16
Alanine	91	2.55±0.62
Histidine	84	2.35±0.12
Methionine	70	1.96±0.09
Glycine	60	1.67±0.23
Cysteine	21	0.58±0.06
Sum	3504	100
Essential Amino acids	1459	40.69
Branched Chain Amino Acids	667	18.61
Aromatic Amino Acids	294	8.21
Sulphur-containing Amino Acids	91	2.54

Values are means ± SD based on 40 observations; Essential Amino acids; Branched chain amino acids (BCAA-Leu, Ile and Val); Aromatic Amino Acids (AAA- Phe and Tyr); Sulphur-Containing Amino Acids (SAA-Met +Cys*)*

The Ser content in Busha milk was comparable to the other breeds (Holstein Friesian 5.69%, Brown Swiss 5.60% Swedish 5.50%, Czech Fleckvieh 5.19%, and Holstein 5.16%). Busha milk composition was highly different from what Froutan et al (2019) reported on commercial cow's milk, where glutamate, tryptophan, tyrosine, glycine, glutamine, serine, threonine, and taurine were the main amino acid detected. These differences in amino acid composition between breeds can be affected by several factors such as genetic variation within breed, health environment, management practices and diet (Abbaya, Yohanna et al., 2022; Křížová et al., 2013; Rafiq et al., 2016b; Selvaggi et al., 2014; Walker et al., 2004).

Milk proteins constitute a favorable balance of amino acids comprised of essential and non-essential amino acids in varying concentrations. Essential amino acids in Busha breed milk represent 40.69% of the total amino acid content (TAA), making this breed's milk a great resource of proteins with high nutritional value. Branched chain amino acids (BCAA: Leu, Ile, Val) accounted for 18.61% of the TAA composition, aromatic amino acids (AAA: Phe , Tyr) accounted for 8.21% of TAA, while the Sulphur-containing amino acids (SAA: Met +Cys) accounted for 2.54% of the TAA, which show an ideal balance of those amino acids supporting its nutrition value and healthy benefits.

In comparing amino acid profiles between the two strains of Busha cattle, only a few amino acids showed a significant difference ($P < 0.05$). The milk of Dukagjini Busha was found to have higher concentrations of Glu, Cys, Tyr, and His when contrasted with the milk from Sharri Busha (Berisha et al., 2021). The disparities in the milk composition between the two Busha strains could be attributed to several factors, including breed-specific genetic variations, environmental conditions, feeding regimes, and farming practices. Other factors such as lactation stage, number of calvings, seasonal variations, management or production systems, geographical location, and age of the cattle could also play a role (Abbaya, Yohanna et al., 2022; Křížová et al., 2013; Rafiq et al., 2016b; Selvaggi et al., 2014; Walker et al., 2004).

5.1.3. Biogenic amine composition of Busha milk

Among the biogenic amines, only spermine (0.11 mg /kg Sharri Busha; 0.07 mg/kg Dukagjini Busha) and cadaverine (0.18 mg /kg Sharri Busha; 0.15 mg/kg Dukagjini Busha) were detected in small quantities in the milk samples. No significant differences were detected in the biogenic amine content between the milk of the two Busha breeds (Dukagjini and Sharri). Busha milk doesn't represent health risk for the customers based on the BA composition.

5.1.4. Fatty acid composition of Busha milk

The fatty acid composition of Busha cattle milk is shown in table 5. Saturated fatty acids (SFA) were the main fatty acid group, accounting for 56.45% of the total fatty acids. Among SFAs, palmitic acid (C16:0) was the most abundant (26.52%). Monounsaturated fatty acids (MUFA) were also prominent (40.26%). Oleic acid (C18:1) was the main MUFA (38.12%). Polyunsaturated fatty acids (PUFA) were present in 3.29%. Linoleic acid (C18:2) was the major PUFA (2.15%). The milk fat extracted from bovine milk typically constitutes about 70% SFA, 25% MUFA, and 5% PUFA (Shingfield et al., 2008). However, the fatty acid profile of Busha cattle milk deviates from this typical profile, with a lower percentage of SFA and a higher percentage of MUFA. This could be due to a variety of factors, including the breed of the cattle, their diet, and the specific conditions in which they are raised. When comparing Busha cattle fatty acids with various cattle breeds it underscores distinctive nutritional profiles, with the Busha breed demonstrating notable differences. In terms of saturated fatty acids, Busha cattle milk have a substantially lower content at 56.45%, in contrast to Simmental (69.84%), Holstein-Friesian (69.68%), Brown Swiss (70.18%), and Alpine Grey (69.67%) (Gottardo et al., 2017). This discrepancy suggests that the Busha breed may offer a leaner milk option, which aligns with contemporary dietary recommendations emphasizing the health benefits of reducing SFA intake. While monounsaturated fatty acids in Busha cattle present a markedly higher percentage at 40.26%, outpacing Simmental (25.58%), Holstein-Friesian (25.72%), Brown Swiss (24.95%), and Alpine Grey (26.12%) (Gottardo et al., 2017). The elevated MUFA content in Busha meat, particularly oleic acid (C18:1), implies potential cardiovascular advantages for consumers, as MUFAs are associated with heart-healthy properties. Examining polyunsaturated fatty acids, Busha cattle had a slightly higher percentage at 3.29%, in comparison to Simmental (2.88%), Holstein-Friesian (2.92%), Brown Swiss (2.95%), and Alpine Grey (3.19%) (Gottardo et al., 2017). While the differences are marginal, the elevated PUFA content in Busha milk contributes to a more balanced fatty acid profile, enhancing the nutritional value of the milk.

Table 5. Fatty acid composition of Busha cattle milk

Fatty Acid Composition	**%**
Butyric acid C4:0	1.71
Caproic acid C6:0	0.92
C:10 - Capric acid C10:0	0.68
Lauric acid C12:0	0.87
Myristic acid C14:0	5.51
Pentadecanoic acid C15:0	0.76
Palmitic acid C16:0	26.52
Palmitoleic acid C16:1	2.14
Margaric acid C17:0	1.08
Stearic acid C18:0	18.41
Oleic acid C18:1	38.12
Linoleic acid C18:2	2.15
Gamma-linolenic acid (GLA) C18:3n6	0.46
Alpha-linolenic acid (ALA) C18:3n3	0.67
Sum of SFA	56.45
Sum of MUFA	40.26
Sum of PUFA	3.29

*SFA-Saturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids

The overall fatty acid composition revealed a well-balanced profile, with a notable presence of oleic acid and palmitic acid, highlighting the potential nutritional significance of Busha cattle milk. These findings have implications for human nutrition, as the fatty acid composition of milk is known to influence the health properties of dairy products. The high proportion of oleic acid, a beneficial monounsaturated fatty acid, may contribute to the perceived health benefits associated with the consumption of dairy products.

5.2. Traditional Kosovan cheeses composition

5.2.1. Moisture and protein content of traditional cheeses

The comparative analysis of moisture and protein content among different cheese types reveals notable variations in their compositional profiles (Table 6). Moisture content, a critical determinant of cheese texture and mouthfeel, exhibits a descending trend from cow cheese (CC) to Sharri cheese (SC). The moisture of the soft self-fermented cheeses produced from buffalo, cow, and goat milk and of semi-hard cheeses Sharri and Rugova cheese (RC) was within the standards of the Joint FAO/WHO (2023) requirements for standardized soft cheese with a minimum of 67% and semi-hard cheese 45%. Moisture content was the highest in cow soft cheese (CC 77.50%), followed by buffalo cheese (BC 77.35%) and goat cheese (GC 72.74%) among soft cheeses. At the same time, Sharri cheese had lower moisture content compared to Rugova cheese. Sharri and Rugova semi-hard cheeses had higher moisture content compared to well-known semi-hard cheeses Gauda with 39.40-42.86% (Jo et al., 2018; Jung et al., 2013) and Cheddar cheese 36.15-36.99% (Guinee et al., 2006).

The protein content was the highest in GC (19.79%), followed by CC (17.13%) and SC (15.64%), while RC (12.02%) and BC (10.72%) had the lowest protein content detected (Table 6). The protein content detected in the goat cheese samples was higher than reported in the studies by Rahayu & Kusnandar, (2011) and Setyawardani et al., (2017) for goat cheeses, which reported values of 15.67% and 10.76%, respectively. Conversely, Mustafa et al. (2013)showed a lower protein content of 14.57%, while Owni & Hamed (2009) identified a higher protein content of 22.50% in cow cheese compared to this study findings in cow cheese samples. Regarding buffalo cheese, Shahein et al. (2014)identified a higher protein content of 14.40% than what was observed in buffalo cheese samples. The relatively lower protein content in buffalo cheese in our study could be attributed to the specific breeding practices in Kosovo, where buffalos are primarily raised for enhanced fat production. Rugova and Sharri cheeses had lower protein content compared to semi-hard cheeses such as Cheddar, which had a protein content range of 26.00-26.37% (Guinee et al., 2006), as well as Gouda, which had reported values of 24.22% (Indumathi et al., 2015) and 23.80% (Sulieman et al., 2018).

Table 6. Moisture and protein content of traditional cheeses

Cheese type	Moisture content (%)	Protein content (%)
Buffalo cheese	77.35 ±0.60	10.72 ±0.06
Cow cheese	77.50 ±0.50	17.13 ±0.15
Goat cheese	72.74 ±0.30	19.79 ±0.05
Rugova cheese	50.62±1.35	12.01±2.30
Sharri Cheese	47.67±2.03	15.64±2.57

Results are shown as mean value ±standard deviation n=9

Table 7 shows the amino acid composition of various traditional cheeses. Statistical analysis (MANOVA) revealed significant differences ($p < 0.05$) in some of amino acids among the five different types of cheese, as presented in Table 7. The amino acid concentration was highest in goat cheese (212.18 mg/g). Cow cheese had a concentration of 185.08 mg/g, Sharri cheese had a concentration of 157.80 mg/g. Buffalo and Rugova cheeses contained 109.08 mg/g and 103.71 mg/g, respectively, which is half the quantity of goat cheese. The main amino acids present in cheeses were glutamic acid (BC 24.95%; CC 24.88%; RC 24.82%; GC 24.16%; SC 23.46%), proline (GC 17.22%; CC 16.10%; BC 15.06%; RC 12.99%; 8.48%), leucine (RC 10.22%; BC 9.87%; GC 9.41%; SC 8.92%; CC 8.74%), lysine (SC 7.80%; RC 6.88%; CC 6.73%; BC 6.19%; GC 5.80%) and aspartic acid (SC 7.65%; CC 6.14%; RC 6.23%; GC 5.53%; BC 5.65%). The content of glutamic acid, which is a prevalent amino acid, is consistently high in all cheeses, although Sharri Cheese has a slightly lower percentage. The content of proline varies considerably, with buffalo cheese having the highest concentration and Sharri cheese having the lowest. Buffalo cheese, goat cheese, and Rugova show similarities in their leucine levels, compared to cow and Sharri cheese. The lysine content in goat cheese was slightly lower compared to other cheese types.

The essential amino acids accounted for about 40% (SC 42.09%; RC 39.80%; BC 39.48%; GC 37.53%; CC 35.98%) of the total amino acid content in these cheeses, highlighting their significance as a protein source with exceptional nutritional value (Figure 7). The concentrations of phenylalanine and threonine in Rugova cheese differed significantly from those in other types of cheese. More precisely, Rugova cheese had lower levels of Phe, while demonstrating a much higher concentration of Thr compared to the other types of cheese (Table 7). The concentration of methionine varied significantly, with buffalo cheese having the highest quantity (2.80%), while cow cheese and goat cheese have relatively lower values (0.53% and 0.50%, respectively). Arginine and histidine were similar across various types of cheeses, except Sharri Cheese, which contains a higher quantity of arginine compared to other cheese types. Additionally, buffalo cheese showed lower levels of histidine compared to other cheeses. Sharri cheese was the best cheese when considering the content of essential amino acids compared to other types of cheeses. Rugova and buffalo cheeses, despite having a protein level of around 11%, demonstrate superior protein quality when compared to goat and cow cheese. This is attributed to their higher proportions of essential amino acids. These results shed important light on the essential amino acid profiles of various kinds of cheese and have implications for their nutritional content and possible health advantages.

The diverse amino acid compositions found in the analyzed traditional cheeses have important implications for their food quality and nutritional value. It is interesting to note that these cheeses are produced by non-professionals using traditional cheese-making technology that has been passed down from generation to generation, demonstrating the potential of traditional methods to produce foods with excellent nutritional profiles. Furthermore, the diverse range of essential amino acids found in these cheeses, which make up a significant proportion of the overall amino acid content, highlights their nutritional significance.

Table 7. Protein building amino acids composition of different cheeses.

Cheese Type	**Buffalo Cheese**		**Cow Cheese**		**Goat cheese**		**Rugova Cheese**		**Sharri Cheese**	
Amino Acids	mg /g	%	mg /g	%	mg /g	%	mg /g	%	mg /g	%
Glu	27.21	24.95	46.04	24.88	51.26	24.16	25.74	24.82	37.03	23.46
Pro	16.42	15.06	29.80	16.10	36.55	17.22	13.47	12.99	13.39	8.48
Leu	10.76	9.87	16.17	8.74	19.97	9.41	10.60	10.22	14.07	8.92
Lys	6.75	6.19	12.45	6.73	12.20	5.75	7.14	6.88	12.40	7.86
Asp	6.17	5.65	11.37	6.14	11.74	5.53	6.46	6.23	12.07	7.65
Val	5.64	5.17	8.80	4.75	12.31	5.80	4.87	4.69	5.62	3.56
Ser	5.38	4.94	9.76	5.28	10.79	5.09	5.16	4.97	11.03	6.99
Phe	4.23	3.88	7.47	4.04	9.57	4.51	3.05	2.94	7.19	4.56
Thr	4.07	3.73	6.45	3.49	8.12	3.83	6.08	5.87	6.88	4.36
Tyr	4.06	3.72	8.82	4.77	9.84	4.64	3.94	3.80	7.08	4.49
Ile	3.63	3.33	5.04	2.72	5.86	2.76	2.61	2.51	5.82	3.69
Met	3.05	2.80	0.98	0.53	1.07	0.50	2.27	2.19	5.07	3.22
Arg	2.84	2.60	5.18	2.80	5.40	2.55	2.55	2.46	5.39	3.42
Ala	2.50	2.30	4.63	2.50	4.17	1.96	1.68	1.62	6.63	4.20
Cys	2.40	2.20	4.42	2.39	5.15	2.43	4.17	4.02	0.00	0.00
His	2.10	1.92	4.04	2.18	5.13	2.42	2.11	2.04	3.97	2.52
Gly	1.87	1.71	3.65	1.97	3.04	1.43	1.81	1.75	4.15	2.63
Total Amino Acid	109.08	100.00	185.08	100.00	212.18	100.00	103.71	100.00	157.80	100.00

Data are shown as mean values; n = 9.

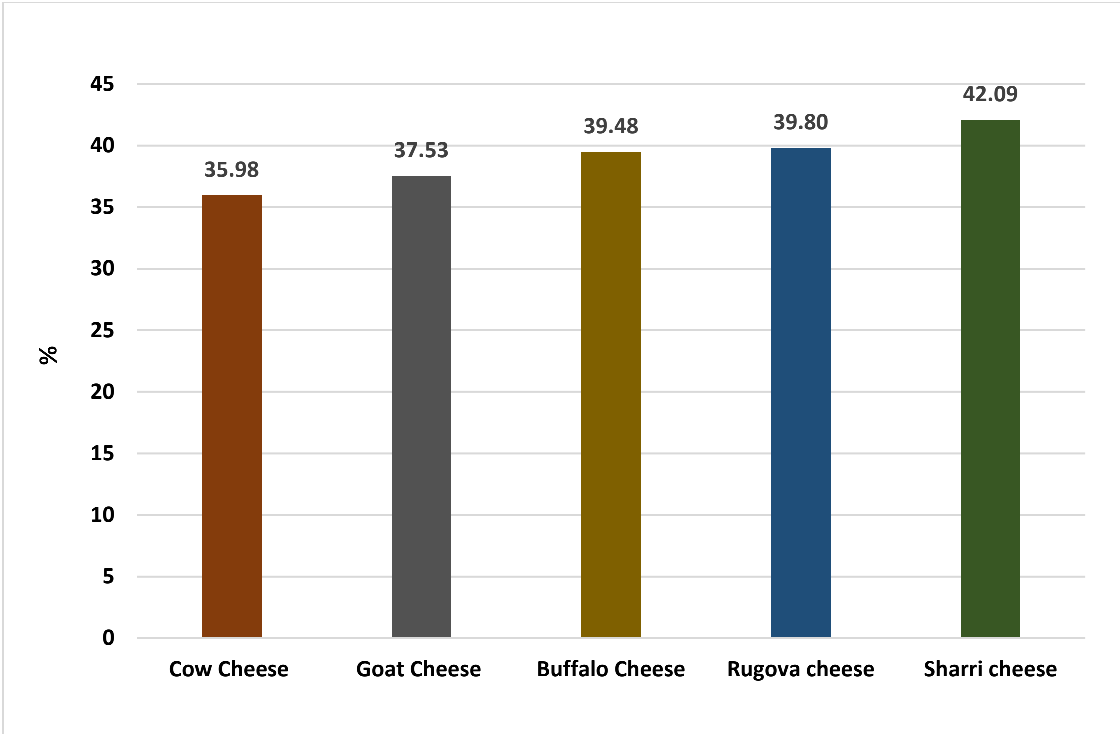

Figure 7. Essential amino acid content of different types of cheeses

The data was evaluated using Principal Component Analysis (PCA) to examine the distribution of the samples according to the type of cheese. Two primary components, PC1 and PC2, described 98.96% of the overall variation. PC1 accounted for 88.40% of the variance, while PC2 accounted for 10.56% (Figure 8). PCA segregated cheeses into distinct clusters depending on their amino acid composition. The buffalo and Rugova cheeses were positioned in the negative quadrant of PC2 (Figure 8). The disparities in amino acid composition between cow and goat are small mostly may be affected by the variances in the content of Leu, Lys, Asp, Val and Ala. Sharri cheese is situated apart on the positive side of PC2. The Sharri cheese contained low amount of Pro and Asp and much higher Lys and Arg, while Cys wasn't detected. The variations in the amino acid content across different types of cheese can be attributed to several factors, including the type of milk, the microbial cultures used during the cheese-making process, and the aging method. These variables can influence the proteolysis of casein, the main protein found in milk, and result in the synthesis of different amino acids. The variations in amino acid composition can also impact the texture, flavor, and aroma of the cheese. High concentrations of Glu and Pro can enhance the umami flavor and structural stability of cheeses.

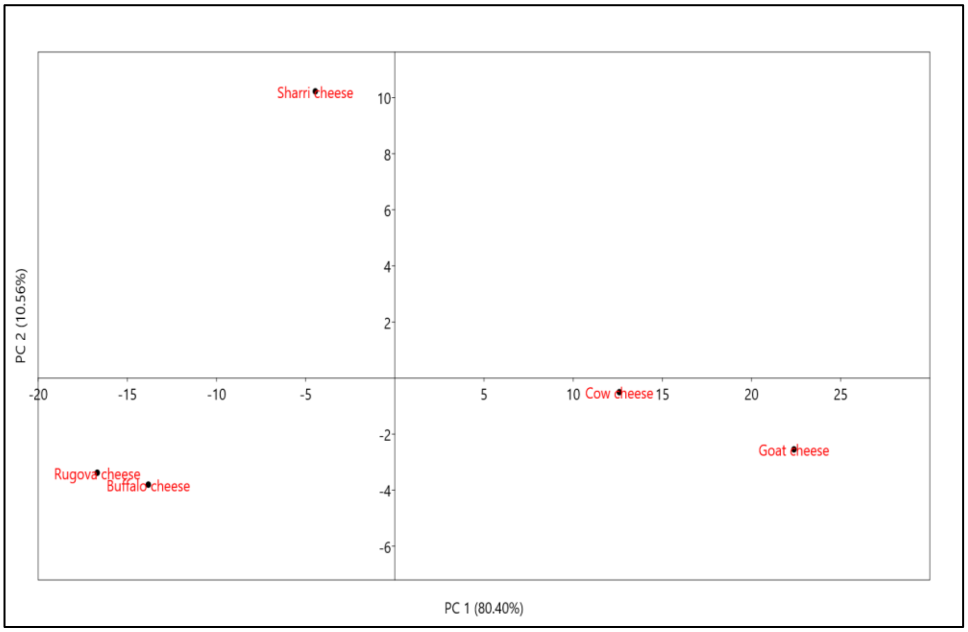

Figure 8.. Classification of cheeses by principal component analysis based on the protein building amino acids

5.2.2. Free amino acid composition of traditional cheeses

Table 8 shows the free amino acid (FAA) composition of traditional cheeses. The main free amino acids detected in buffalo cheese were GABA, Glu, Lys, Pro, and Asp, collectively accounting for 61.83% of the total free amino acid content (TFAA). The main amino acids found in cow cheese were GABA, Lys, Pro, Met, Orn, and Ala, accounting for 63.83% of the TFAA. The main free amino acid in goat and Sharri cheeses accounted for around 57% of the total free amino acids. In goat cheese, the main FAA were GABA, Leu, Glu, Pro, and Ala, whereas in Sharri cheese, the main FAA were GABA, Ile, Tyr, Asp, and Val. The main FAAs found in Rugova cheese were GABA, Leu, Lys, Glu, and Pro, which accounted for 61.88% of the TFAA.

GABA (gamma-aminobutyric acid) is the most abundant FAA in all cheeses (SC 24.44%; RC 21.18%; CC 20.82%; BC 19.84%; GC 18.29%). Several studies have shown that GABA on plays a crucial role in the human body, reducing blood pressure, promoting relaxation, enhancing the immune system's response to stress, suppressing long-term alcohol-related illnesses, and inhibiting cancer cell growth (Abdou et al., 2006; Ramos-Ruiz et al., 2018; Simon Sarkadi, 2019). Studies have shown that hypertensive patients who consume fermented milk containing 10-12 mg of GABA daily for 12 weeks experience a decrease in blood pressure (Inoue et al., 2003). Traditional Spanish cheeses rich in GABA have also been found to reduce blood pressure (Diana et al., 2014). Considering that those cheeses are highly consumed in Kosova, daily consumption of these cheeses will contribute to their beneficial effects on health.

Leucine is found in high amounts in goat cheese and Rugova cheese, making up 16.64% and 14.08% of their composition, respectively. In comparison, Sharri cheese has a relatively lower level of leucine at 1.65%. Leucine, known for its role in enhancing flavor, can contribute to the overall taste experience of these cheeses. The relatively lower level of leucine in Sharri cheese may influence its sensory profile differently, potentially resulting in a distinct flavor profile compared to the other cheeses. Glutamic acid is highest in buffalo cheese (12.77%) and significantly lower in Sharri cheese (0.23%). Proline levels are relatively consistent among cheeses, except Rugova cheese which shows a lower concentration at 1.18%. Methionine and phenylalanine concentrations vary among the cheeses. Cow cheese has the highest Met content (7.13%), while Sharri and cow cheese has the highest Phe content (5.73% respectively 5.38%). Also, lysine (Lys) is notably higher in buffalo (12.79%), cow (12.66%) and Rugova Cheese (11.45%) compared to Sharri (4.47%) and goat (2.16%) cheeses. The variations in glutamic acid, proline, methionine, phenylalanine, and lysine levels among the cheeses contribute to the overall taste and nutritional composition. These differences highlight the diversity in sensory attributes, allowing consumers to choose cheeses based on their individual preferences and dietary needs.

Except for Rugova Cheese, all other cheeses lack Alpha-Aminoadipic acid. Furthermore, it is important to mention that in Sharri cheese glutamine, serine and cystathionine weren't detected. Moreover, Sharri Cheese stands out due to its unique free amino acid profile, which includes the sole presence of Citrulline and Arginine. These findings improve our comprehension of the complex variations in amino acid profiles, offering useful insights for the sensory evaluation and nutritional assessment cheeses.

Branched-chain amino acid (BCAA: leucine, isoleucine, and valine) content was highly different in cheeses (Figure 9). Goat cheese had the highest amount of BCAA (25.00 %) followed by Rugova and Sharri cheese (18.57% respectively 18.28%). Cow cheese (10.06%) and buffalo cheese (8.75%) had significantly lower BCAA content. 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 (Gleeson, 2005; Simon Sarkadi, 2019). The observed differences in BCAA content indicated that the nutritional composition of cheese strongly depends on the type of cheese.

Table 8. Free amino acids composition of different cheeses (%)

Free Amino Acids	Buffalo cheese	Cow cheese	Goat cheese	Rugova cheese	Sharri cheese
Gamma-aminobutyric acid	19.84^{ab}	20.82^{b}	18.29^{a}	21.18^{b}	24.44^{c}
Leucine	3.56^{a}	2.13^{b}	16.64^{c}	14.08^{c}	1.65^{b}
Glutamic acid	12.77^{a}	2.64^{b}	9.62^{c}	8.02^{c}	0.23^{d}
Proline	9.34^{a}	9.57^{a}	6.79^{b}	7.15^{b}	1.18^{c}
Alanine	2.99^{a}	6.73^{b}	6.61^{b}	2.69^{a}	2.79^{a}
Methionine	5.62^{a}	7.13^{b}	5.14^{a}	3.65^{c}	1.45^{d}
Phenylalanine	1.99^{a}	2.93^{b}	5.38^{c}	1.85^{a}	5.73^{c}
Valine	2.46^{a}	4.22^{b}	5.21^{cd}	1.99^{a}	6.68^{d}
Asparagine	3.61^{a}	1.21^{b}	4.17^{a}	3.81^{a}	0.00^{c}
Threonine	1.19^{a}	1.08^{a}	3.63^{b}	1.17^{a}	5.70^{c}
Histidine	3.82^{a}	1.40^{b}	3.24^{a}	1.78^{b}	1.84^{b}
Aspartic acid	7.09^{a}	4.46^{b}	3.22^{b}	4.65^{b}	7.37^{a}
Isoleucine	2.73^{a}	3.71^{b}	3.16^{b}	2.50^{a}	9.95^{c}
Glutamine	0.79^{a}	0.37^{a}	2.98^{b}	0.40^{a}	0.00^{c}
Lysine	12.79^{a}	12.66^{a}	2.16^{b}	11.45^{a}	4.47^{c}
Serine	2.65^{a}	3.72^{b}	1.50^{c}	3.26^{b}	0.00^{c}
Ornithine	1.86^{a}	6.92^{b}	0.78^{c}	1.08^{a}	3.52^{d}
Glycine	0.40^{a}	0.78^{a}	0.82^{a}	2.99^{b}	1.79^{b}
Tyrosine	2.19^{a}	3.66^{b}	0.54^{c}	1.92^{a}	9.42^{d}
Cysteine	1.29^{a}	2.46^{b}	0.16^{c}	1.06^{a}	3.15^{b}
Cystathionine	1.02^{a}	1.41^{a}	0.00^{b}	2.51^{c}	0.00^{b}
Alpha-Aminoadipic acid	0.00^{a}	0.00^{a}	0.00^{a}	0.79^{b}	0.00^{a}
Citrulline	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	5.98^{b}
Arginine	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	2.68^{b}

Data are shown as mean $n = 9$; Data of buffalo, cow, and goat cheese (Berisha et al., 2023).

Cheeses had a high concentration of aromatic amino acids (AAA: phenylalanine and tyrosine), which is best known for their role in the nervous system. The highest amount of AAA was detected in SC (15.15%) followed by CC (6.59%), GC (5.91%), BC (4.18%) and RC (3.77%) (Figure 8).

The analysed cheeses are a good source of sulphur-containing amino acids (Met+Cys: CC 9.59%; BC 6.92%; GC 5.30%; RC 4.71%; SC 4.60%), which serve important biological functions. Methionine and cysteine maintain normal cellular functions and health and are known for their antioxidant effect. In countries with a plant-based diet, especially those with high consumption of legumes (e.g., Kosovo), consumption of this cheese in addition to legumes helps satisfy the Recommended Dietary Allowance (RDA) for sulphur-containing amino acids (SAA), because the amount of SAA in legumes is very low.

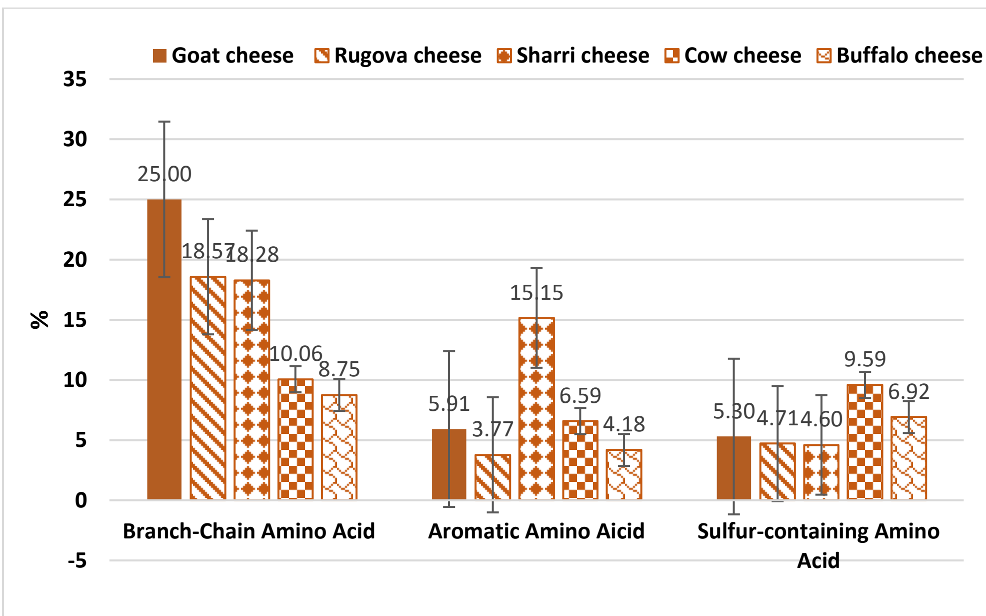

Figure 9. Branch chain amino acids (BCAA), aromatic amino acids and sulfur-containing amino acids in traditional cheeses,

Traditional cheeses in Kosovo have diverse free amino acid (FAA) compositions that not only contribute to their nutritional value but also shape their sensory properties. Those cheeses, rich in BCAA content, AAA, and SAA, not only offer nutritional benefits but also contribute to the culinary scene. The complex interaction of amino acid compositions in these cheeses demonstrates the durability and efficiency of traditional cheese-making techniques. These findings highlight the potential influence of these cheeses on human health and sensory perceptions. As the food industry evolves, it is crucial to prioritize the conservation and recognition of traditional production techniques to ensure their continued importance in health and cultural heritage.

Principal Component Analysis (PCA) showed good separation of cheese based on the free amino acid composition. Two principal components (PC) accounted for 83.81% of the total variance: PC1 52.52%; PC2 31.29% of the total variance (Figure 10). PC1 highlighted distinct differences, placing goat and Sharri cheeses on the positive side of PC2, opposite to Buffalo and Cow cheeses, which were positioned on the negative side of PC1. Rugova Cheese occupied the edge of the positive side of PC2. This spatial distribution aligns with the specific variations in the FAA composition of these cheeses. For instance, Sharri Cheese stood out for its elevated GABA content and unique presence of citrulline and arginine, as identified in the FAA analysis. Additionally, the lower levels of leucine in Sharri cheese compared to goat cheese further contribute to the separation observed in the PCA. The higher concentrations of glutamic acid in buffalo cheese and distinctive amino acid profiles in cow cheese, such as elevated methionine and phenylalanine. further substantiate the PCA results. These findings underscore the intricate relationship between PCA outcomes and the specific FAA compositions of cheeses, providing valuable insights for both sensory evaluation and nutritional assessment.

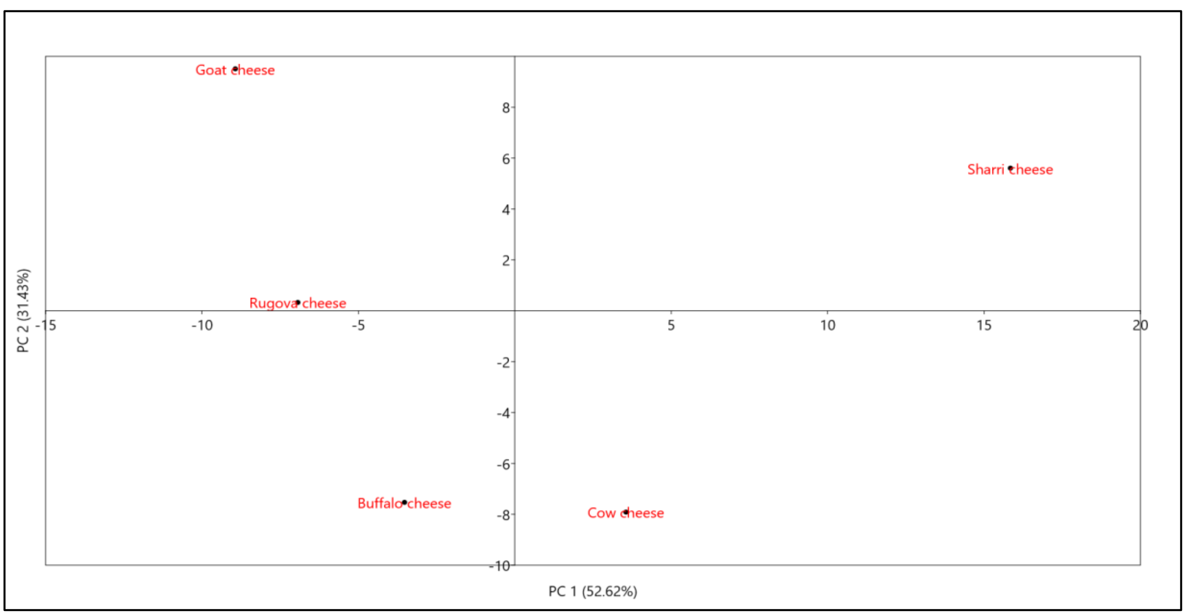

Figure 10.. Classification of traditional cheeses by principal component analysis (PCA) based on the free amino acids

5.2.3. Essential amino acids score of traditional cheeses

Nutritional requirements, including both protein and amino acids, undergo dynamic changes across various life stages. In early life, characterized by rapid growth and intense development, the demand for essential amino acids is notably heightened. Histidine, traditionally considered semi-essential or conditionally essential in adulthood, assumes an essential status during childhood, necessitating adequate intake. Accordingly, the FAO/WHO reference protein is tailored to specific age groups: 0-6 months, 6 months to 3 years, and beyond 3 years, optimizing the essential amino acid ratios. Although protein requirements evolve with growth, physical activity, and individual needs, the demand for essential amino acids remains relatively stable over time (Food and Agriculture Organization Expert Working Group, 2018). Thus, our analysis focused on comparing amino acid compositions of cheese proteins with the WHO reference proteins for age groups 3-14 years, 15-18 years, and 18+. To assess the quality of each essential amino acid, the mg/g protein content in each sample was divided by the corresponding mg/g protein content of the FAO/WHO reference protein, yielding the Essential Amino Acid Score (EAAS). A score nearing 1 for each essential amino acid signifies alignment with the FAO/WHO reference protein composition. A ratio below 1 designates the limiting amino acid, with the results and identified limiting amino acids presented in Table 9.

Table 9. Essential Amino Acid Score (EAAS) of traditional cheeses based on the age groups

Cheese Type	Age	Leucine	Lysine	Valine	Phenylalanine and Tyrosine	Threonine	Isoleucine	Methionine and Cysteine	Histidine
Buffalo Cheese	3-14 Years	1.62	1.29	1.29	1.85	1.49	1.11	2.17	1.20
	15-18 Years	1.64	1.32	1.29	1.90	1.55	1.11	2.17	1.20
	>18 Years	1.67	1.38	1.33	2.00	1.62	1.11	2.27	1.28
Cow Cheese	3-14 Years	1.43	1.40	1.19	2.15	1.39	*0.91*	1.27	1.36
	15-18 Years	1.46	1.43	1.19	2.20	1.45	*0.91*	1.27	1.36
	>18 Years	1.48	1.50	1.22	2.32	1.52	*0.91*	1.33	1.46
Goat Cheese	3-14 Years	1.54	1.20	1.45	2.23	1.53	*0.92*	1.27	1.51
	15-18 Years	1.57	1.22	1.45	2.29	1.59	*0.92*	1.27	1.51
	>18 Years	1.60	1.28	1.49	2.41	1.66	*0.92*	1.33	1.61
Rugova Cheese	3-14 Years	1.46	1.64	*0.89*	2.21	1.74	1.23	1.40	1.57
	15-18 Years	1.49	1.67	*0.89*	2.26	1.82	1.23	1.40	1.57
	>18 Years	1.51	1.75	*0.91*	2.38	1.90	1.23	1.46	1.68
Sharri Cheese	3-14 Years	1.68	1.43	1.17	1.65	2.35	*0.84*	2.70	1.27
	15-18 Years	1.70	1.46	1.17	1.69	2.44	*0.84*	2.70	1.27
	>18 Years	1.73	1.53	1.20	1.78	2.55	*0.84*	2.82	1.36

Leucine, lysine, phenylalanine and tyrosine, threonine, methionine and cysteine, and histidine demonstrated satisfactory EAAS values across all cheeses and age groups, with EAAS above 1, suggesting no limiting amino acids. For isoleucine, cow, goat, and Sharri cheeses essential amino acid score fell below 1, designating isoleucine as a limiting amino acid, in all age groups of those types of cheeses. Valine is considered limited in Rugova cheese. These findings underscore nuanced differences in amino acid compositions among cheese types and age groups, providing valuable insights into dietary considerations and potential areas for refining protein quality in the analyzed cheeses.

5.2.4. Essential amino acid deficiency index in traditional cheeses

The Essential Amino Acid Deficiency Index was used to evaluate the amino acid profiles of various cheeses across different age groups, based on their essential amino acid score. Since the total essential amino acid score for leucine, lysine, phenylalanine and tyrosine, threonine, methionine and cysteine, and histidine were above 1, their essential amino acid deficiency ratio is 0. Such in the calculation of the essential amino acid deficiency index they give negative values, showing surplus of essential amino acids compared to the reference protein. Moderate deficiencies were observed across all age groups in isoleucine in cow, goat and Sharri cheese. Rugova Cheese demonstrated moderate deficits in valine. These findings provide detailed insights into the amino acid compositions of the analyzed cheeses, pinpointing specific deficiencies and surpluses and informing strategies for dietary optimization.

5.2.5. Biogenic amines in traditional cheeses

Biogenic amines were detected in low concentration only in goat soft cheese and Rugova cheese (Table 10). Tyramine (544 µg/g) and histamine (468 µg/g) were the main amines followed by cadaverine (102 µg/g) and putrescine (78 µg/g) in goat cheese. While Spermidine (299 µg/g) and Spermine (245 µg/g) were the main BAs detected in the Rugova cheese. 0.11 mg /kg Sharri Busha; 0.07 mg/kg Dukagjini Busha. Although biogenic amine (BA) formation is often associated with long-aged and hard cheeses, emerging evidence shows that short-ripened and soft cheeses can also accumulate substantial BA levels, particularly when made from raw milk or under spontaneous fermentation. Many microorganisms especially decarboxylase-positive

Enterococcus, Lactobacillus, and Lactococcus species can convert free amino acids into biogenic amines even within relatively short maturation periods (Natrella et al., 2024). In goat cheeses produced from unpasteurized milk, higher indigenous microbial load and lack of starter culture control facilitate BA accumulation (Novella-Rodriguez et al. 2004). A study quantifying BA in goat milk cheeses further supports that soft or semi-soft goat cheeses do indeed contain measurable levels of amines, consistent with variation in microbial activity and cheese microenvironment (Kandasamy et al., 2021). Additionally, a recent review on dairy BA formation identifies pH, salt content, proteolysis, and microbial gene expression as critical modulators of BA accumulation in dairy products (including soft cheeses) (Dankar et al., 2025). Finally, molecular evidence shows that bacteria isolated from dairy products often harbor histidine/tyrosine decarboxylase genes, reinforcing that endogenous microbial enzymes are central to BA formation in cheeses (Shanab et al., 2025). In goat cheese, self-fermentation of raw milk plus the favorable presence of decarboxylase-active microflora likely enabled BA formation even during a short ripening period.

According to EFSA regulations (2011), the biogenic amine content of these cheeses does not represent a health risk to consumers.

Table 10. Biogenic amine composition of traditional cheese samples

Biogenic amine	**Buffalo cheese (μg/g)**	**Cow cheese (μg/g)**	**Goat cheese (μg/g)**	**Rugova Cheese (μg/g)**	**Sharri Cheese (μg/g)**
Histamine	ND	ND	468±12	126±9	ND
Tyramine	ND	ND	544±38	157±7	ND
Cadaverine	ND	ND	102±13	103±9	ND
Putrescin	ND	ND	78±5	97±6	ND
Spermine	ND	ND	ND	245±18	ND
Spermidine	ND	ND	ND	299±19	ND

Data are shown as mean ± standard deviation; n = 9; ND: not detected.

5.2.6. Fatty acid composition of traditional cheeses

The proportion of each fatty acid group in their profiles varied depending on the type of milk used for cheesemaking (Table 11). 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) resulted in the greatest differences among the fatty acids identified. The highest proportions of caprylic acid (9.63%), myristic acid (11.61%), and palmitic acid (30.47%) were found in goat cheese, followed by cow cheese (1.24% C10:0; 3.33% C14:0; 25.04% C16:0) and buffalo cheese. Buffalo cheese had significantly higher levels of stearic acid (37.63%) and linoleic acid (19.75%) than cow cheese (19.61% C18:0; 3.15% C18:2) or goat cheese (10.82% C18:0; 3.32% C18:2). Cow cheese had the highest oleic acid content (33.39%), followed by goat cheese (19.14%) and buffalo cheese (10.51%).

Saturated fatty acids (SFA) were the main group in soft cheeses, with goat cheese accounting for 74.45% of the total fatty acid content. Monounsaturated fatty acids (MUFA) were the highest in cow cheese (37.25%), followed by goat cheese (21.08%), and buffalo cheese (13.66%). Buffalo cheese had a significantly higher polyunsaturated fatty acid (PUFA) content than goat (3.96%) or cow cheese (7.51%). Linoleic acid was the primary PUFA in buffalo cheese (19.75%) The high level of PUFA in buffalo soft cheese indicates that this Kosovan buffalo breed has good fat quality

The fatty acid composition analysis of Rugova and Sharri cheeses reveals interesting insights into their lipid profiles. Both cheeses have similar saturated fatty acid (SFA) compositions, with palmitic acid (C16:0) and stearic acid (C18:0) being the dominant SFAs. These SFAs contribute significantly to the overall solidity and texture of the cheeses.

However, the cheeses differ in their monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) profiles. Rugova Cheese has a higher proportion of MUFA (RC 38.24%; 26.08%), primarily due to a higher content of oleic acid (C18:1), which is known for its positive impact on cardiovascular health. On the other hand, Sharri Cheese exhibits a higher PUFA content, with linoleic acid (C18:2) and alpha-linolenic acid (C18:3) being the main contributors. This higher PUFA content may impact the nutritional profile and potential health benefits of the cheese.

Table 11. Fatty acid composition of buffalo, cow, and goat soft cheeses

Fatty acids	Buffalo cheese (%)	Cow cheese (%)	Goat cheese (%)	Rugova Cheese (%)	Sharri Cheese (%)
Butyric acid C4:0	1.55[a]	2.46[b]	1.51[a]	2.03[b]	1.72[a]
Caproic acid C6:0	2.15[a]	1.40[b]	1.73[b]	0.93[c]	1.87[b]
Capric acid C8:0	2.24[a]	1.72[b]	3.35[c]	2.11[a]	2.36[a]
Caprylic acid C10:0	1.26[a]	1.24[a]	9.63[b]	1.75[a]	1.23[a]
Lauric acid C12:0	2.32[a]	ND	5.33[b]	1.80[c]	2.75[a]
Myristic acid C14:0	1.94[a]	3.33[b]	11.61[c]	5.57[d]	8.07[e]
Palmitic acid C16:0	11.02[a]	25.04[b]	30.47[c]	26.9[b]	24.97[b]
Stearic acid C18: 0	37.63[a]	19.61[b]	10.82[c]	17.07[bd]	15.41[d]
Palmitoleic acid C16:1	2.55[a]	3.86[b]	1.94[c]	2.11[a]	1.73[c]
Oleic acid C18:1	10.51[a]	33.39[b]	19.14[c]	36.13[d]	24.35[e]
Linoleic acid C18:2	19.75[a]	3.15[b]	3.32[b]	2.12[c]	11.44[d]
Alfa-linolenic acid C18:3	6.85[a]	4.36[b]	0.64[c]	1.48[d]	4.10[b]
Unknown	0.23[a]	0.44[a]	0.51[a]	ND	ND
Sum SFA	60.11[a]	54.80[b]	74.45[c]	58.16[d]	58.38[d]
Sum MUFA	13.06[a]	37.25[b]	21.08[c]	38.24[b]	26.08[d]
Sum PUFA	26.60[a]	7.51[b]	3.96[c]	3.60[c]	15.54[d]

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 Principal Component Analysis (PCA) results reveal substantial separation among various cheeses based on their fatty acid composition (Figure 11). The two principal components, PC1 and PC2, collectively account for 96.47% of the total variance (PC1 74.76%; PC2 21.71%) The positioning of cheeses in the PCA space further elucidates the distinct fatty acid profiles of each type. Goat cheese, characterized by elevated levels of caprylic acid (C10:0), myristic acid (C14:0), and palmitic acid (C16:0), occupies a unique position on the positive side of PC1, indicating substantial compositional differences from other cheeses. Cow and Rugova cheese, closely positioned on the negative side of PC1, stands out for their high oleic acid (C18:1) content. Buffalo cheese, located at the edge of the negative side of PC2, distinguishes itself with higher stearic acid (C18:0) and linoleic acid (C18:2) levels. These findings underscore the critical role of specific fatty acids in shaping the unique characteristics of each cheese type, as reflected in their distinct positions within the PCA space.

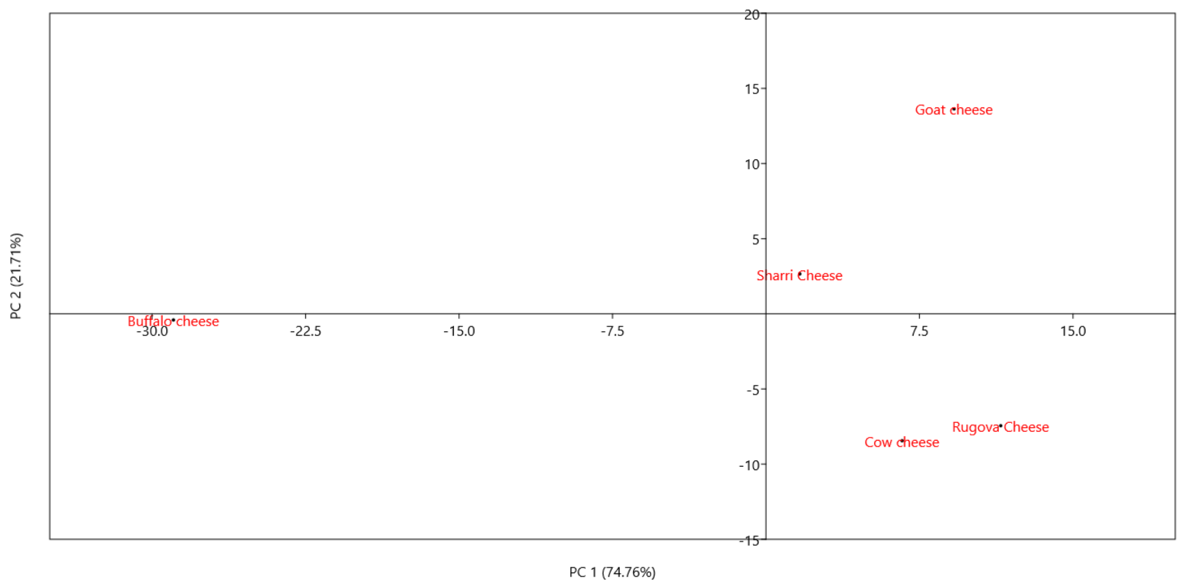

Figure 11. Classification of cheeses by principal component analysis (PCA) based on the fatty acid composition

5.3. Composition of traditional beef meat products

5.3.1. Moisture, protein and fat content of traditional dry beef meat products

The moisture, protein, of traditional beef ham, dry beef meat and beef sausage is shown in Table 12. The moisture content in dry beef meat (44.86%) was higher than that reported by Dincer and Erbas in 2018 and 2019 (32.11% and 39.76-39.90%, respectively), but it was lower than the 48.39% observed in dried beef slices by Başlar et al. (2014). Additionally, the protein content in dry beef meat was significantly higher at 49.00% compared to the literature value of 22.89% (Başlar et al., 2014). The moisture content of traditional beef sausage (31.28%) was comparatively lower than that of Australian retail raw beef sausage samples ranging from 58.8% to 70.2%

(Cunningham et al., 2015). The protein content of the homemade beef sausage was higher (36.74%) than both the Australian retail beef sausages (11.60-17.00%) and dry fermented beef sausages (19.33-26.51%) analyzed by (Olivares et al., 2011). To our knowledge this study presents the initial data on the moisture and protein content of beef ham.

Table 12. Moisture, protein content of traditional beef ham, dry beef meat and beef sausage

Composition	Beef Ham	Dry Beef Meat	Beef Sausage
Moisture content (%)	52.25±2.36	44.86±4.10	31.28±2.50
Protein (%)	44.12±3.65	49.00±0.36	36.74±1.95

Data are given as mean±sd- standard deviation (n=9)

5.3.2. Protein-building amino acid composition of traditional dry beef meat products

The total amino acid (TAA) content of protein-building amino acids of traditional dry beef meat, beef ham and beef sausage were 469.71 mg/kg, 427.22mg/kg and 344.92 mg/kg, respectively (Table 13). The main amino acids detected in traditional dry beef meat included Glu (17.55%), Lys (10.83%), Asp (8.82%), Arg (7.62%), and Ala (6.25%) with 60.36% of TAA. In the traditional beef ham main amino acids accounted for 63.39% with Glu (15.50%), Asp (13.47%), Leu (9.12%), Arg (8.52%), Thr (8.46%) and Lys (8.32%). The major amino acids in traditional beef sausage were Ala (15.56%), Leu (13.28%), Gly (8.64%), Pro (8.41%), Ser (8.26%), and Val (7.65%) accounted for 61.80% of the TAA content. Cysteine (0.81%) was the minor amino acid. The amino acid profile of beef traditional products is important in determining nutritional value and potential health effects. In general, amino acid concentrations in beef sausage are lower than in traditional beef dry meat and beef ham, and of beef meat showed by (Jorfi et al., 2012). The amino acid profile of sausages can vary depending on factors such as manufacturing method and other ingredients used (Aro Aro et al., 2010; Bär et al., 2020; Domínguez et al., 2016). The observed differences in total amino acid composition between the traditional beef sausage and the other two products (dry beef and beef ham) can be attributed to both technological processing and raw material origin. Traditional sausages were produced using a mixture of lean beef and added fat (approximately 70% lean meat and 30% fat), whereas dry beef and ham were prepared from pure muscle cuts with lower lipid content. This formulation difference inherently alters the protein-to-fat ratio and consequently the amino acid concentration per gram of product. Moreover, the thermal drying of sausage at higher temperatures (80 °C for 12 h) induces partial protein denaturation, oxidation, and Maillard reactions, which may reduce recoveries of thermolabile amino acids such as lysine, cysteine, and tryptophan after hydrolysis. In contrast, dry beef and ham undergo milder air-drying or curing processes that better preserve amino acid integrity. Similar effects of heat treatment and fat content on amino acid composition have been reported in studies describing protein and amino acid degradation during roasting and fermentation (Xia et al., 2021; Wang et al., 2022).

Table 13. Protein-building amino acid composition of traditional beef ham, dry beef meat and beef sausage

Meat type	Beef Ham		Dry Beef Meat		Beef sausage	
Amino acids	**mg/g**	**%**	**mg/g**	**%**	**mg/g**	**%**
Glutamic acid	66.24	15.50^{a}	82.45	17.55^{b}	13.83	4.01^{c}
Lysine	35.56	8.32^{a}	50.85	10.83^{b}	19.92	5.78^{c}
Leucine	38.95	9.12^{a}	43.62	9.29^{a}	45.80	13.28^{b}
Aspartic acid	57.55	13.47^{a}	41.43	8.82^{b}	16.90	4.90^{c}
Arginine	36.38	8.52^{a}	35.81	7.62^{b}	13.33	3.86^{c}
Alanine	18.23	4.27^{a}	29.37	6.25^{b}	53.66	15.56^{c}
Glycine	23.76	5.56^{a}	26.69	5.68^{a}	29.79	8.64^{b}
Threonine	36.15	8.46^{a}	21.07	4.49^{b}	9.63	2.79^{c}
Proline	22.98	5.38^{a}	20.51	4.37^{b}	29.00	8.41^{c}
Serine	15.25	3.57^{a}	19.90	4.24^{b}	28.50	8.26^{c}
Histidine	14.24	3.33^{a}	19.17	4.08^{b}	14.53	4.21^{b}
Valine	14.43	3.38^{a}	19.00	4.04^{b}	26.39	7.65^{c}
Phenylalanine	14.33	3.35^{a}	18.82	4.01^{b}	14.29	4.14^{b}
Tyrosine	12.45	2.91^{a}	16.96	3.61^{b}	17.62	5.11^{c}
Isoleucine	12.92	3.02^{a}	15.36	3.27^{a}	4.40	1.27^{b}
Methionine	1.34	0.31^{a}	2.69	0.57^{a}	4.54	1.32^{b}
Cysteine	6.45	1.51^{a}	6.02	1.28^{a}	2.79	0.81^{b}
Total Amino Acid	427.22	100.00	469.71	100.00	344.91	100.00

Data are shown as mean values; (n=9). ; ^{a,b,c} different letters show statistically significant differences ($p < 0.05$)

The MANOVA test of protein-building amino acid composition of traditional dry beef meat, beef ham and beef sausage show significant variations. Traditional dry beef meat and beef ham have significantly higher Glu and Lys compared to beef sausage, suggesting potential differences in flavor and umami characteristics. Leucine, associated with muscle protein synthesis, is higher in beef sausage at 13.28% compared to beef ham and dry beef meat. Alaine content is significantly elevated in beef sausage at 15.56%, suggesting differences in energy metabolism and potential influences on flavor. Threonine, essential for protein synthesis, is highest in traditional beef ham (8.46%), followed by traditional dry beef meat (4.49%) and beef sausage (2.79%). Methionine, crucial for sulfur-containing amino acids, is highest in beef sausage at 1.32%, suggesting potential differences in sulfur-containing amino acid intake and physiological functions.

According to the results traditional beef meat products are an excellent source of essential amino acids, accounting for more than 40 % of the TAA composition (Figure 12). The highest essential amino acids content was detected in dry beef meat (48.20%), followed by traditional beef ham (47.82%) and traditional beef sausage (44.31%). Those data show that traditional beef meat products are a good source of proteins with high biological value.

The amino acid profile of traditional beef products is crucial for their nutritional value, as essential amino acids are essential for various physiological functions like protein synthesis and immune system support. The diverse amino acid profiles in these products contribute to meeting these requirements, making them valuable additions to a balanced diet. Understanding regional and cultural influences on these products is essential, as they can offer alternatives to those with dietary restrictions or preferences, enhancing the diversity of protein sources. Therefore, the amino acid profiles of traditional beef products highlight their nutritional richness and importance in a balanced diet.

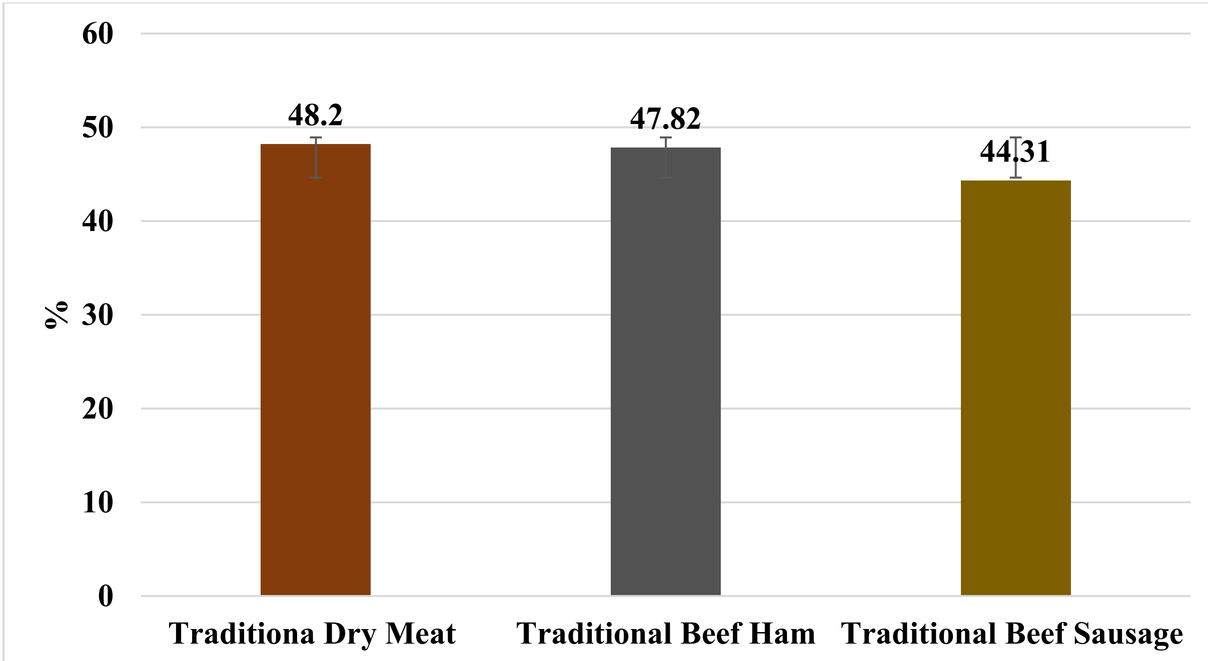

Figure 12. Essential Amino Acid content of Traditional Dry Beef Meat, Beef Ham and Beef Sausage

The PCA results provide valuable insights into the protein-building amino acid composition of traditional dry beef meat, beef ham, and beef sausage. The analysis reveals a clear separation among these products, with two principal components (PC1 82.72% and PC2 11.20) explaining 99.92% of the total variance (Figure 13). Traditional dry beef meat occupies the positive side of PC2, indicating a distinct amino acid profile contributing to the variability along this component. This positioning aligns with the high concentrations of Glu, Lys, and Ala observed in dry beef meat. On the other hand, traditional beef ham is situated on the negative side of PC1, opposing the X-axis. The separation along PC1 indicates that beef ham exhibits distinct amino acid patterns compared to other products. The higher concentrations of Glu, Asp, and Leu, contribute to its position in the PCA plot. Traditional beef sausage, positioned at the edge of the negative side of PC2, suggests a specific amino acid profile that distinguishes it from the other products. The PCA results align with the lower amino acid concentrations observed in beef sausage compared to traditional dry beef meat and beef ham. The PCA results, supported by the MANOVA test, highlight the unique amino acid compositions of traditional dry beef meat, beef ham, and beef sausage. These distinctions contribute to variations in flavor, nutritional value, and potential health effects, providing valuable information for consumers and researchers interested in the diverse characteristics of traditional beef products.

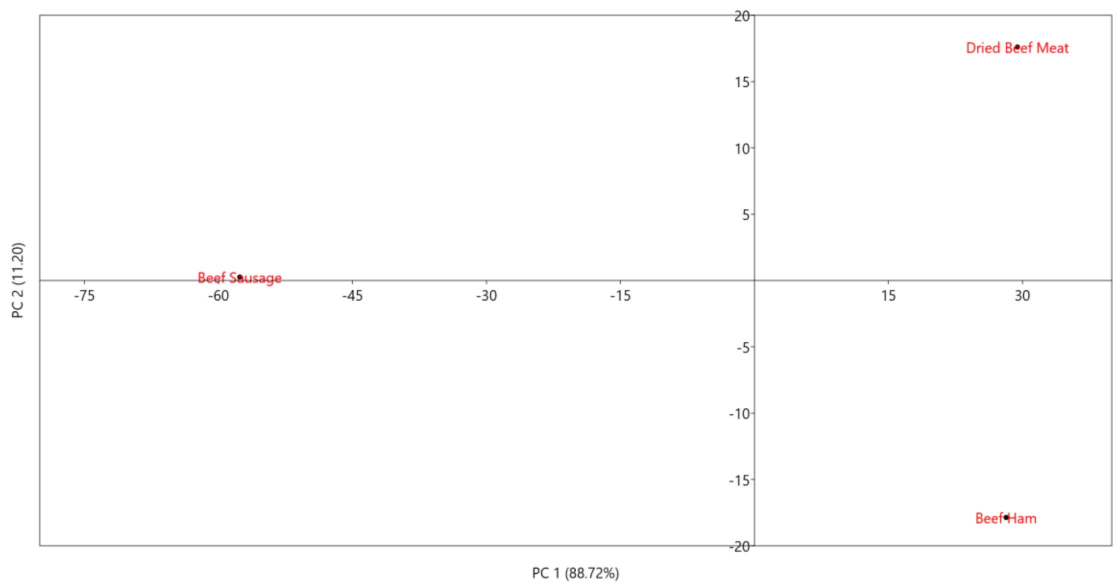

Figure 1. Classification of traditional beef meat products by principal component analysis (PCA) based on the protein building amino acid composition

5.3.3. Free amino acid composition of traditional dry beef meat products

In addition to the protein-building amino acids, the free amino acid (FAA) composition of a food product is also an important aspect that determines its quality (Table 14). Main free amino acids detected in dry beef meat were Glu (11.19%), Gln (10.01%), Lys (8.86%), Asp (8.80%), Leu (7.98%), Arg (5.97%) and Pro (5.58%), with 58.39% of the total FAA. Ala (11.06%), Asp (10.79%), Glu (10.60%), Gln (9.78), Lys (8.44%), and Gly (6.00%) were the main FAA of traditional beef ham with 56.67% of total FAA. The most abundant FAAs were Ala (22.81%), Glu (11.62%), Leu (9.20%), and Lys (7.68%) accounting for more than 50% of the total FAA.

This is similar to the findings of (Gallego et al., 2018), who investigated the free amino acid profile of European dry-fermented pork sausages and identified Ala, Glu, Leu, and Lys as the most abundant amino acids. Kononiuk et al. (2020) found that fermented sausages made from beef and fallow deer meat with sour whey contained higher concentrations of certain amino acids including Gly, Pro, and Val, than homemade beef sausages. Domínguez et al. (2016) investigated the effect of starter cultures on the free amino acid content of dry-cured foal sausage and found that different starter cultures cause large differences in amino acid composition. FAAs also play an important role in sensory qualities e.g., glutamic acid and aspartic acid contributes to the fresh flavour, glycine and alanine contribute to the sweet taste, arginine, leucine, valine, and phenylalanine contribute to bitter taste, and lysine contributes to both sweet and bitter taste (Mau & Tseng, 1998).

Table 14. Free amino acid composition of traditional dry beef meat, beef ham and beef sausage

Meat type and amino acid	Traditional beef ham (%)	Traditional dry beef meat (%)	Traditional beef sausage (%)
Glutamic acid	10.6	11.19	2.09
Glutamine	9.78	10.01	11.62
Lysine	8.44	8.86	7.68
Aspartic acid	10.79	8.80	1.65
Leucine	6.66	7.98	9.2
Arginine	4.50	5.97	3.22
Proline	2.57	5.58	1.50
Cystathionine	4.68	4.93	0.67
Alanine	11.06	4.87	22.81
Serine	4.57	4.61	3.79
Threonine	4.72	4.53	4.29
Glycine	6.00	4.40	5.61
Valine	3.07	4.40	4.31
Phenylalanine	3.56	3.48	1.74
Tyrosine	2.84	3.34	3.54
Histidine	3.51	3.31	4.29
Isoleucine	2.19	2.75	3.89
Methionine	0.30	0.83	3.44
Cysteine	0.16	0.16	0.22
Ornithine	0	0	4.44

Data are shown as mean values; (n=9).

The nutritional evaluation of traditional beef sausage revealed distinctive amino acid characteristics compared with other beef products such as dry beef meat and beef ham. According to Figure 14, sausage exhibited markedly higher concentrations of branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs), while sulfur-containing amino acids were present at relatively lower levels. This pattern is nutritionally relevant, as BCAAs particularly leucine, isoleucine, and valine are crucial for muscle protein synthesis and energy metabolism, whereas AAAs such as phenylalanine and tyrosine contribute to neurotransmitter biosynthesis and flavor development. The elevated levels of these amino acids in sausage may reflect both the raw material composition and the processing method, which concentrates free amino acids during fermentation and curing.

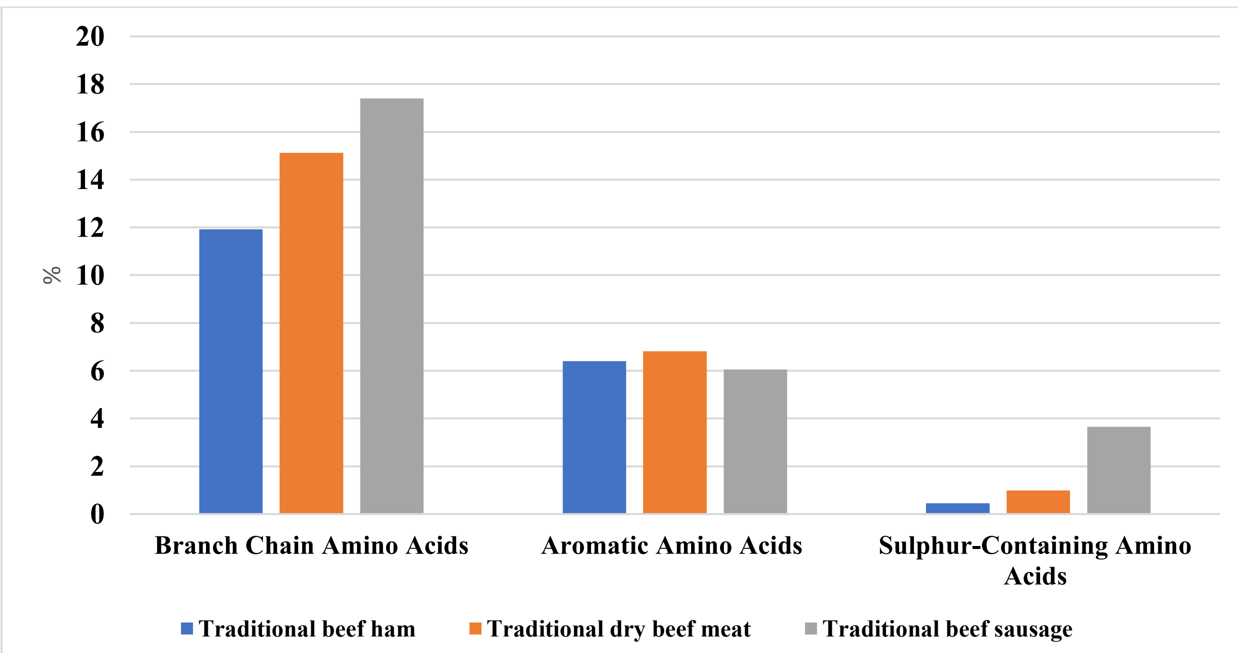

Figure 2. Branch chain amino acids, aromatic amino acids and sulfur-containing amino acids in traditional beef meat products

The PCA results provide valuable insights into the free amino acid composition of traditional dry beef meat, beef ham, and beef sausage. The analysis reveals a clear separation among these products, with two principal components (PC1 = 89.51% and PC2 = 10.49%) together explaining 100% of the total variance (Figure 15). Traditional dry beef meat is positioned on the positive side of PC1 and the upper quadrant of PC2, indicating that its amino acid profile strongly contributes to the variability captured by both components. This separation aligns with the relatively high concentrations of histidine, lysine, and phenylalanine + tyrosine observed in dry beef meat. In contrast, traditional beef ham is situated on the positive side of PC1 but on the negative side of PC2, reflecting a distinct amino acid pattern compared to dry beef meat. Its positioning may be explained by the higher levels of threonine, leucine, and methionine, which distinguish ham from the other beef products. Traditional beef sausage, however, occupies the far negative side of PC1, suggesting a markedly different free amino acid profile. This separation highlights the influence of fermentation and processing conditions in shaping its composition, with elevated branched-chain amino acids (particularly leucine and valine) but a limiting level of isoleucine.

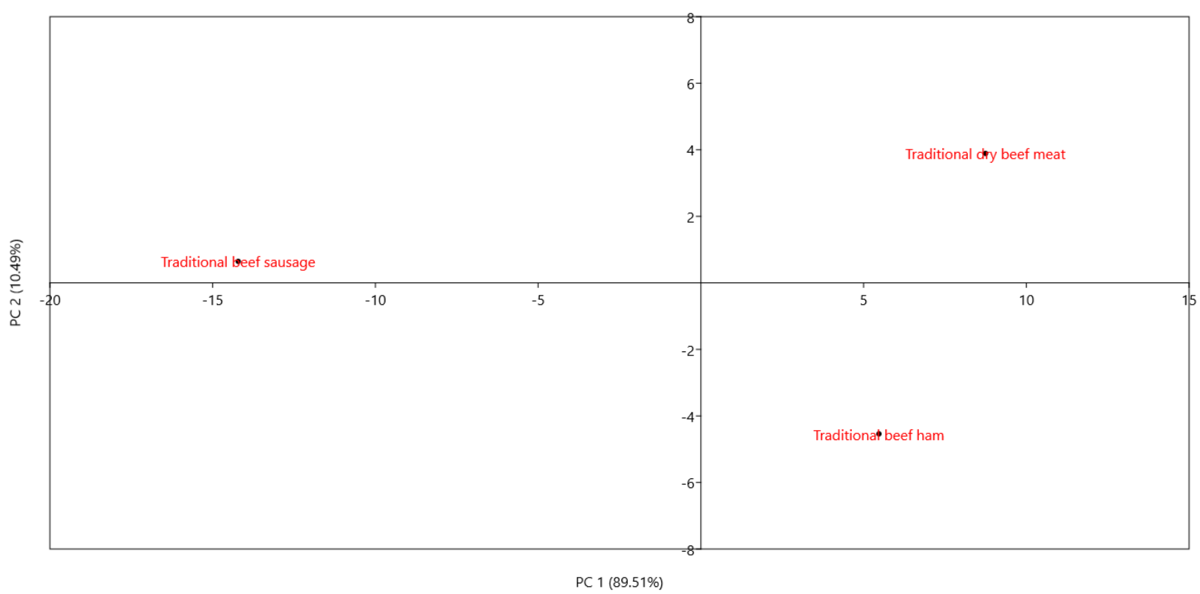

Figure 35. Classification of traditional beef meat products by principal component analysis (PCA) based on the free amino acid composition

The Essential Amino Acid Score (EAAS) across age groups (Table 15) provided further insights into the nutritional adequacy of sausage. For children (3–14 years), the EAAS values indicated that leucine (2.18), valine (1.91), phenylalanine + tyrosine (2.26), and histidine (2.63) were well above the reference requirement, confirming sausage as a valuable source of these essential amino acids. However, isoleucine (0.42) and methionine (0.92) were limiting factors, with isoleucine falling considerably below the threshold for all age groups. This imbalance persisted in adolescents and adults, suggesting that while sausage contributes significantly to certain essential amino acids, it cannot be regarded as a complete protein source on its own. Nevertheless, when consumed as part of a mixed diet, sausage provides high-quality protein enrichment, particularly for muscle-related amino acids such as leucine and valine.

Table 15. Essential Amino Acid Score (EAAS) of traditional beef meat products based on the age groups

Beef meat product	Age group	Lys	Leu	Thr	His	Val	Phe+ Tyr	Ile	Met
Traditional Beef Ham	3-14 Years	1.69	1.46	3.31	2.04	0.83	1.49	0.99	1.77
	15-18 Years	1.73	1.48	3.44	2.04	0.83	1.53	0.99	1.77
	>18 Years	1.81	1.51	3.59	2.17	0.85	1.61	0.99	1.85
Traditional Dry Beef Meat	3-14 Years	2.21	1.49	1.76	2.50	0.99	1.82	1.07	1.70
	15-18 Years	2.26	1.52	1.83	2.50	0.99	1.86	1.07	1.70
	>18 Years	2.36	1.54	1.91	2.66	1.02	1.96	1.07	1.77
Traditional Beef Sausage	3-14 Years	1.20	2.18	1.12	2.63	1.91	2.26	***0.42***	0.92
	15-18 Years	1.23	2.21	1.16	2.63	1.91	2.31	***0.42***	0.92
	>18 Years	1.28	2.25	1.21	2.81	1.96	2.43	***0.42***	0.97

5.3.4. Biogenic amine composition of traditional dry beef meat products

Table 16 shows the biogenic amine (BA) content of homemade beef sausage. The total concentration of biogenic amines was 1.69 mg/kg. Histamine, agmatine and tyramine were the most abundant biogenic amines, with concentrations of 1.03, 0.28 and 0.16 mg/kg respectively. Spermine (Spm) and spermidine (Spd) concentrations of 0.04 and 0.03 mg kg-1 were also detected. The BA content of homemade beef sausages was within the range reported by (Kononiuk & Karwowska, 2020b) in beef dry fermented sausages (1.7 - 2.1 mg/kg). However, the amine profile found in homemade beef sausage differs from what was reported in that study, with histamine being the most abundant rather than putrescine. BA contents in homemade beef sausages were significantly lower than those reported in traditional European pork sausages, where tyramine, putrescine, and cadaverine were the most detected (Latorre-Moratalla et al., 2008). Other studies found significantly higher levels of Spm and Spd in foal sausage made with various starter cultures (Domínguez et al., 2016). Several factors influence the formation of biogenic amines in food, including microbial population growth and activity, amino acid decarboxylase activity, starter culture, and fermentation process (Roseiro et al., 2010; Schirone et al., 2022). The low concentration of biogenic amines in homemade beef sausages could be explained by a lack of starter culture or fermentation.

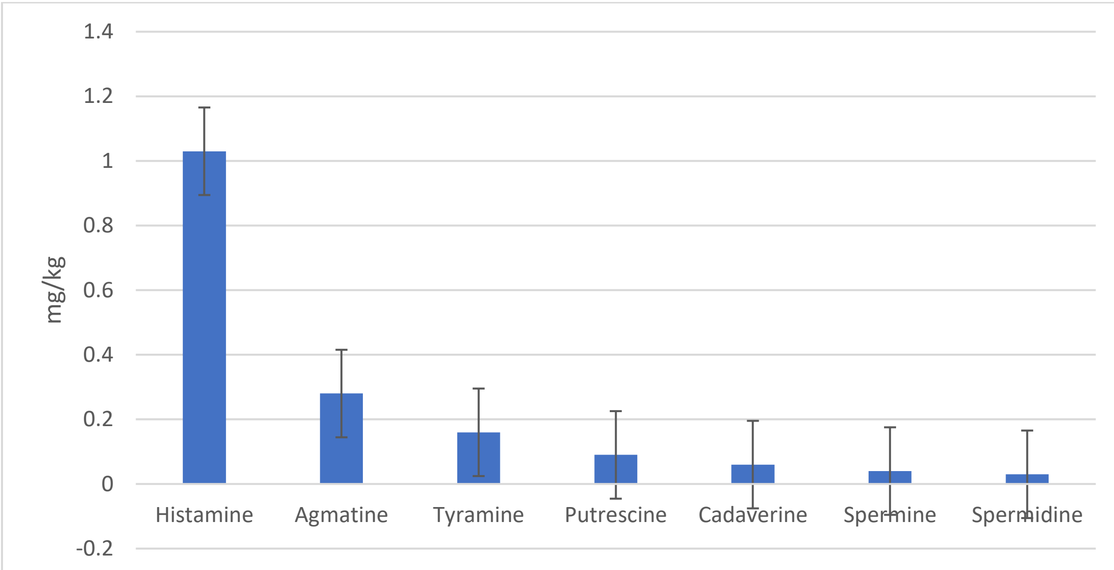

Figure 4. Biogenic amine composition of homemade beef sausage

5.3.5. Fatty acid composition of homemade beef sausage

The fatty acid profile of the homemade beef sausages is shown in Figure 16. The homemade beef sausages contained a total of 13 different fatty acids, with oleic acid (C18:1) accounting for 34.37%, followed by palmitic acid (C16:0; 30.24%), and stearic acid (C18:0) accounting for 18.78%. Short chain saturated fatty acids (SCFA) such as butyric acid (C4:0; 1.16%), caproic acid (C6:0; 0.20%), and capric acid (C8:0; 0.18%) were also present in lower concentrations in the sausages. The SCFA are well-known for their role in colon physiology, acting as energy sources for host cells and the intestinal microbiota, and taking part in various host-signalling mechanisms (Ríos-Covián et al., 2016).

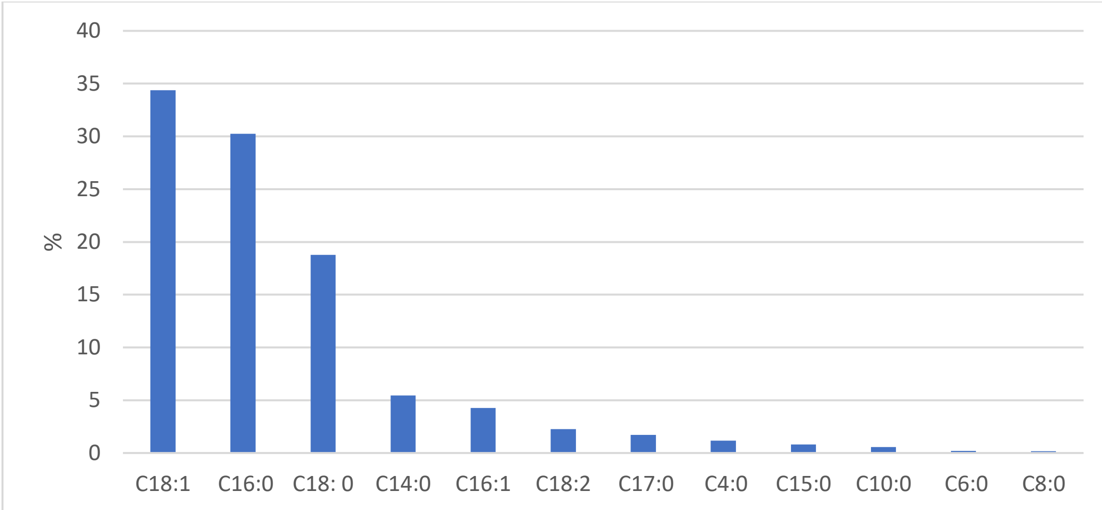

Figure 5. Fatty acid composition of homemade beef sausage

The unsaturated fatty acids found in the homemade beef sausage included 34.37% oleic acid (C18:1) and 2.27% linoleic acid (C18:2). According to emerging scientific evidence, the type of fatty acids in the diet plays a crucial role in determining our health outcomes. Oleic acid has been shown to have a variety of health benefits, including lowering blood pressure and oxidative stress (Shen et al., 2022). On the other hand, linoleic acid, a polyunsaturated fatty acid, is considered an essential fatty acid because it cannot be synthesised by the body and must be obtained through diet. Recent research suggests that linoleic acid may protect against heart disease and improve cognitive function by acting as a precursor to important compounds such as prostaglandins and leukotrienes that are involved in inflammation and immune function regulation (Belury, 2023). The homemade beef sausages contained high amount of saturated fatty acids (59.10%) and monounsaturated fatty acids (38.63%), but only low amount of polyunsaturated fatty acids (2.27%). The unsaturated to saturated fatty acid ratio was 0.69, indicating that the homemade beef sausages were high in saturated fatty acids and low in monounsaturated and polyunsaturated fatty acids.

6. CONCLUSIONS AND RECOMANDATIONS

The present study represents the first comprehensive nutritional characterization of Busha milk, traditional Kosovan cheeses, and traditional beef products, providing novel information into their amino acid, biogenic amine, and fatty acid composition. The analysis of Busha milk confirmed its distinctive nutritional profile, particularly the high content of essential amino acids and a favorable fatty acid balance. These results underline the scientific and cultural importance of the Busha breed, which, despite its endangered status, offers significant nutritional value. Preserving this breed should therefore be regarded not only as an act of biodiversity conservation but also as a contribution to maintaining high-quality nutritional resources for future generations.

The determination of essential amino acid scores and deficiency indexes revealed that Busha milk possesses superior quality compared to several commercial dairy sources. This indicates its potential to play a key role in diversifying dietary options in Kosovo and beyond. Similarly, the comparative evaluation of traditional cheeses demonstrated substantial differences among varieties, with Rugova and Sharri cheeses emerging as particularly rich sources of essential amino acids. These findings highlight the functional food potential of certain cheese types, positioning them as valuable contributors to human health and nutrition. At the same time, the study of biogenic amines in cheeses revealed elevated concentrations of histamine and tyramine in some varieties, raising important food safety concerns. This underscores the need for regular monitoring and the establishment of quality control protocols to safeguard consumers without undermining traditional production practices.

The fatty acid composition of traditional cheeses was found to be generally favorable, with a higher proportion of unsaturated fatty acids, supporting the growing evidence that traditional dairy products may exert health-promoting effects when consumed responsibly. Likewise, the characterization of traditional beef products provided the first scientific profile of their nutritional value, revealing that homemade beef sausages possess higher levels of free amino acids and beneficial fatty acid ratios than other meat products. This suggests that these traditional products, when properly controlled for safety, may serve as high-quality sources of protein and essential nutrients. Taken together, these findings establish the first nutritional database for traditional foods of Kosovo, contributing not only to food science but also to cultural heritage preservation.

Based on these results, several recommendations can be made. Conservation and breeding programs for the Busha cattle breed should be strengthened to secure its survival and to preserve its unique milk composition. Food safety regulations need to incorporate systematic monitoring of biogenic amines in traditional cheeses, as elevated levels pose potential risks to consumers. At the same time, producers should be encouraged to adopt improved processing practices that reduce biogenic amine formation while maintaining sensory quality. Another important factor as the promotion of traditional cheeses, especially Rugova and Sharri varieties, as health-promoting foods could increase their market value and contribute to the economic sustainability of local producers. Consumer awareness campaigns should also be launched to emphasize both the nutritional benefits and the potential risks associated with traditional foods, thereby supporting informed dietary choices.

Further research is recommended to expand the scope of this study to other traditional Kosovan foods, to investigate seasonal and regional variations in composition, and to explore innovative technologies that ensure food safety without compromising traditional authenticity. The nutritional database established in this dissertation should be maintained and expanded, serving as a foundation for future scientific investigations, consumer education, and policymaking in food safety, nutrition, and cultural heritage preservation.

7. NEW SCIENTIFIC RESULTS

1. I found that Busha cattle milk, particularly from the Dukagjini strain, had significantly higher dry matter, fat, and lactose contents, making it nutritionally superior within the breed. I demonstrated that its fat and protein levels are comparable to or higher than those of international high-yielding breeds, while its amino acid profile, with notably elevated glutamic acid and proline, highlights its exceptional protein quality. I found that Busha milk contains a healthier fatty acid composition, with lower saturated and higher monounsaturated fatty acids, especially oleic acid, offering potential cardiovascular benefits. I also showed that the very low levels of biogenic amines confirm its safety and high quality. These results are important because they emphasize the genetic and nutritional value of preserving the Busha cattle population, not only as cultural heritage but also as a source of dairy products with potential health-promoting properties for modern consumers.

2. I found that the traditional soft cheeses of Kosovo, produced from buffalo, cow, and goat milk, have distinct nutritional profiles. I demonstrated that goat cheese had the highest protein content (19.79%) and the richest essential amino acid concentration, while cow cheese also showed a strong balance of proteins and amino acids, confirming their high nutritional value compared to international reports. I proved that all soft cheeses contained high levels of glutamic acid and proline, supporting both their structural stability and umami flavor. I also showed that they were particularly rich in health-promoting compounds such as GABA, with cow and goat cheese providing notable levels that are associated with blood pressure reduction and stress regulation. Furthermore, I demonstrated that the fatty acid composition of these soft cheeses was unique: cow cheese was especially rich in oleic acid, goat cheese had higher caprylic and palmitic acids, and buffalo cheese provided elevated stearic and linoleic acids, making each type nutritionally distinct. Importantly, biogenic amine levels were low, confirming that these traditionally produced cheeses are safe for human consumption.

3. I demonstrated that the semi-hard cheeses, Rugova and Sharri, also carry important nutritional benefits, with clear differences between them. I proved that Sharri cheese provided the highest proportion of essential amino acids (42.09%), particularly lysine and arginine, making it a superior protein source compared to many international cheeses like Cheddar and Gouda. Rugova cheese, while lower in overall protein, still demonstrated high-quality protein composition with threonine enrichment and balanced amino acid scores, highlighting its nutritional significance. I also found that their fatty acid profiles were highly distinctive: Rugova cheese was especially rich in oleic acid and monounsaturated fatty acids, suggesting cardiovascular benefits, while Sharri cheese contained higher polyunsaturated fatty acids, including linoleic and alpha-linolenic acids, further supporting its role as a functional food. The presence of elevated GABA and other free amino acids in both cheeses enhances their potential health benefits and sensory diversity. These findings are important because they prove that traditional Sharri and Rugova cheeses are not only culturally valuable but also nutritionally competitive with internationally recognized cheeses, positioning them as promising dairy products for both local consumption and broader markets.

4. I found that traditional Kosovan beef products, including dry beef meat, beef ham, and beef sausage, are nutritionally rich and distinct in composition. I demonstrated that dry beef meat and beef ham had exceptionally high protein contents (49.00% and 44.12%, respectively) and superior essential amino acid proportions (>47%), exceeding values reported in international studies, while beef sausage, though lower in protein (36.74%), contained higher leucine and alanine, which are important for muscle synthesis and energy metabolism. I proved that free amino acid profiles varied significantly, with dry beef meat rich in glutamic acid and lysine, beef ham dominated by alanine and aspartic acid, and beef sausage containing elevated alanine and leucine, all contributing to flavor complexity and nutritional diversity. I also showed that biogenic amines were detected only in very low amounts, confirming product safety, while the fatty acid profile of beef sausage revealed high oleic acid (34.37%), a heart-healthy monounsaturated fatty acid, alongside palmitic and stearic acids. These findings are important because they demonstrate that traditional beef products of Kosovo are not only safe but also provide proteins of high biological value, essential and functional amino acids, and favorable fatty acid components, supporting both their nutritional relevance and their cultural heritage value.

8. SUMMARY

This dissertation provides the first comprehensive nutritional characterization of traditional foods of Kosovo, focusing on Busha cattle milk, artisanal cheeses, and beef products. These foods constitute an important element of Kosovo's cultural heritage and daily diet, yet scientific evaluation of their nutritional quality and safety has been scarce. The study aimed to analyze their amino acid composition, biogenic amine content, and fatty acid profiles, thereby generating a nutritional database to support public health, food safety, and cultural preservation.

Busha cattle milk demonstrated high nutritional quality, with dry matter contents ranging from 13.09–13.86%, protein around 3.7%, and fat levels up to 4.5% in Dukagjini strains. The amino acid profile was rich in glutamic acid and proline, while the fatty acid composition showed lower saturated and higher monounsaturated fatty acids, particularly oleic acid, supporting potential cardiovascular benefits. Biogenic amines were detected only in trace amounts, confirming product safety.

Traditional cheeses showed substantial variability. Goat cheese showed the highest protein content (19.79%) and essential amino acid concentration, while Sharri cheese provided the greatest essential amino acid proportion (42.09%), surpassing international cheeses such as Cheddar and Gouda. Rugova cheese, though lower in protein, was enriched with threonine and oleic acid, highlighting functional value. All cheeses contained notable levels of GABA, a bioactive amino acid associated with stress regulation, while biogenic amine levels remained low, confirming their safety.

Traditional beef products also proved nutritionally valuable. Dry beef meat and beef ham contained very high protein contents (49.00% and 44.12%, respectively), with essential amino acids exceeding 47%. Free amino acid profiles revealed distinct signatures: glutamic acid and lysine dominated in dry beef, alanine and aspartic acid in beef ham, and alanine plus leucine in sausage, contributing to flavor and muscle-synthesis benefits. Fatty acid analysis of beef sausage showed high oleic acid (34.37%) alongside palmitic and stearic acids, while biogenic amines were present at very low levels, confirming safety.

In conclusion, the results demonstrate that Kosovo's traditional foods provide proteins of high biological value, beneficial fatty acids, and safe levels of biogenic amines, confirming both their nutritional and cultural importance. The study recommends conservation of the Busha breed, routine monitoring of biogenic amines in artisanal dairy and meat products, and consumer education on the health-promoting potential of these foods. These findings establish a scientific foundation for strengthening food safety regulations, supporting rural livelihoods, and enhancing the recognition of traditional Kosovan foods in both local and international markets.

9. APPENDICES

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