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EVALUATION OF BIOGENIC AMINES IN GOAT AND SHEEP CHEESES OF SLOVAK ORIGIN

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ABSTRACT

Goat cheese and sheep cheese can offer a range of unique flavors and potential health benefits, making them popular choices among cheese lovers. Both types of cheese contain biogenic amines, which are naturally present in many types of food. The purpose of this study was to assess the levels of the seven most commonly occurring biogenic amines in semi-hard and hard goat and sheep cheeses intended for the Slovak market. The HPLC-DAD method with pre-column derivatization using dansylchloride was employed for the analysis of the hard and semi-hard cheese samples. The presence of biogenic amines was confirmed in all cheese samples, with total levels ranging from 14.4 mg.kg^{-1} to 1322 mg.kg^{-1}. The most abundant compounds found in the samples were tyramine and tryptamine, followed by 2-phenylethylamine and spermine. Histamine was present in 54.5% of the samples, but its concentration did not exceed 68.7 mg.kg^{-1}. Statistically significant differences in the levels of individual biogenic amines were observed between goat and sheep cheeses, except for spermidine and spermine.

Keywords: goat cheese, sheep cheese, biogenic amines, total biogenic amines, HPLC-DAD

INTRODUCTION

Biogenic amines (BA) are compounds containing nitrogen that have a variety of structures (**Hernández-Jover *et al.*, 1997; Durak-Dados *et al.*, 2020**). They can be found in a diverse array of food items such as fish, meat, eggs, cheese, fermented vegetables, fruits, soy products, nuts, beer, wine, and chocolate (**Hernández-Jover *et al.*, 1997; Özogul and Özogul, 2019; Durak-Dados *et al.*, 2020**). Food contains several significant biogenic amines, which include histamine, tyramine, tryptamine, putrescine, cadaverine, and 2-phenylethylamine. These compounds are derived from amino acid precursors, such as histidine, tyrosine, ornithine, lysine, and tryptophan, or phenylalanine in the case of β-phenethylamine. Bacterial microorganisms can promote the formation of these biogenic amines via decarboxylation of amino acids, amination, or transamination of ketones and aldehydes (**Santos, 1996; Özogul and Özogul, 2019**). Biogenic amines impact various cellular processes such as protein and hormone synthesis, as well as alkaloid production. They also affect DNA replication and the permeability of cell membranes. Furthermore, biogenic amines play a role in regulating body functions such as temperature, blood pressure, and brain activity (**Berthold and Nowosielska, 2008**). Biogenic amines are present in all types of foods that contain proteins. They can serve as indicators of freshness and suitability for consumption in certain foods. In some cases, they can enhance the sensory properties of foods. During the maturation process of cheese, aromatic amino acids such as tryptophan, tyrosine, phenylalanine, and histidine can undergo decarboxylation to form corresponding amines like tryptamine, tyramine, 2-phenylethylamine, and histamine, which can impact the aroma and flavour of the cheese (**Fox *et al.*, 2017; Durak-Dados *et al.*, 2020**). Various amines, including histamine, tyramine, cadaverine, putrescine, tryptamine, and phenylethylamine, have been identified in different types of ripening cheeses (**Diaz-Cinco *et al.*, 1992; Linares *et al.*, 2011**). The formation and presence of these compounds in cheese are influenced by various factors, including the availability of substrate, pH, water activity, bacterial density, etc. During cheese fermentation and aging, proteases and peptidases are stimulated, leading to the formation of small peptides and free amino acids. Several factors contribute to the favourable conditions for biogenic amine formation, such as pH levels between 5.0 and 6.5, high water activity between 0.9 and 1.0, and the availability of pyridoxal phosphate, which is a crucial cofactor for amino acid decarboxylase activity. Conversely, high fat content can inhibit the growth of proteolytic bacteria. Optimal temperatures for proteolysis occur during fermentation in the temperature ranging from 25 to 44 °C and cheese ripening in the temperature range between 10 - 20 °C (**Benkerroum, 2016**). The degradation process in food occurs by action of enzymes due to microbial activity or by endogenous tissue activities, this leads to the formation of some amines or increased concentration of those existing in food (**Vinci and Antonelli, 2002**). Furthermore, the biogenic amines concentration in food may serve as hygienic quality indicators for indication of potential spoilage and freshness of food (**Balamatsia *et al.*, 2006; Buňková *et al.*, 2010; Zhang *et al.*, 2015; Costa *et al.*, 2018**).
Many different approaches are used in identification of quality evaluation of food. Important group are sensory parameters that can be evaluated by sensory panel or electronic systems (**Štefániková et al., 2020a; Štefániková et al., 2020b; Brindza et al., 2011)**. Biogenic amines may serve as an important indicator of the hygienic quality of food. The usefulness of biogenic amines as a quality indicator depends on the nature of the product in case the results are more satisfactory in fresh and heat-treated foods than in fermented products (**De la Torre and Conte-Júnior, 2013**). The presence of biogenic amines in food can also pose a risk to public health because of their physiological and toxicological effects. Consuming food that contains high levels of biogenic amines is linked to potential health risks (**Ruiz-Capillas, Herrero, 2019; Özogul and Özogul, 2019**).
An elevated level of biogenic amines in food may indicate non-compliance with hygienic standards during the processing of raw materials, resulting in the presence of unwanted bacteria **(Alberto et al., 2002)**. The presence of biogenic amines in non-fermented foods beyond a certain naturally occurring level is typically considered an indication of potential microbiological contamination **(Durak-Dados et al., 2020)**. Certain biogenic amines, such as histamine and tyramine, are produced through the decarboxylation of amino acids during the regular cheese ripening process, but they are often associated with the growth of harmful bacterial microorganisms. Controlling the microbial load alone is insufficient for accurately assessing food quality, especially regarding the production of biogenic amines **(Triki et al., 2018)**. Histamine and tyramine have the potential to cause health problems in sensitive individuals, highlighting the importance of monitoring their levels in cheese **(O'Callaghan et al., 2017)**. In certain types of cheese, the concentration of biogenic amines may initially increase during the early stages of ripening and subsequently decrease. This pattern can serve as a useful indicator of the level of maturation achieved by these cheeses **(Diaz-Cinco et al., 1992; Rak, 2005)**. To improve the quality and safety of food products, it is crucial to have a comprehensive understanding of the characteristics of biogenic amines, the factors contributing to their production, and their potential effects on consumer health.
Goat and sheep cheeses present a minor group of cheeses in Slovak market, the popularity of these cheeses is increasing, however some cheeses made especially

from sheep milk have a long tradition in Slovakia (e.g. bryndza sheep cheese) **(Semjon et al., 2018a; Semjon et al., 2018b)**.
The primary objective of this study is to evaluate the levels of biogenic amines in hard and semi-hard ripening goat and sheep cheeses made from sheep and goat milk, with the intention of selling them in the Slovak market.

MATERIAL AND METHODS

Reagents and Standard Solutions

Putrescine dihydrochloride (≥98%), cadaverine dihydrochloride (≥98%), 2-phenylethylamine (≥98%), histamine dihydrochloride (≥99%), tyramine hydrochloride (≥99%), spermidine trihydrochloride (≥99%), spermine tetrahydrochloride (≥99%), tryptamine hydrochloride (≥99%) and 1,7-diaminoheptane (≥99%) were purchased from Merck KGaA (Darmstadt, Germany). Hydrochloric acid (35%, p.a.), Sodium carbonate anhydrous powder (p.a.) and ammonium (26%, p.a.) were purchased from Centralchem (Bratislava, Slovakia). The water used for the preparation of standards and eluents and for sample pretreatment was obtained from in-house Milli-Q water purification system (Millipore, France). Dansylchloride (DCl; 1-dimethyl-aminonaphtalene-5-sulfonylchloride, ≥99%) was purchased from Merck KGaA (Darmstadt, Germany). Other solvents 2-propanone (Chromasolv, ≥99%), diethylether (≥99%) and acetonitrile (Chromasolv, ≥99.9%) were from Sigma-Aldrich (Darmstadt, Germany).
A stock solution of individual standards of biogenic amines were prepared by dissolving 100 mg of standard in 100 ml of deionized water, resulting in a concentration of 1 $mg.ml^{-1}$ for each amine standard. The working solution of mixture of standards was prepared by pipetting of 0.5 ml of the individual stock solutions to the volumetric flask and filling up to the volume of 50 ml. Hydrochloric acid (0.1 M) was used as the extraction agent, while the derivatization agent was prepared by dissolving 50 mg of DCl in 10 ml of acetone. Other solutions used in the experiment included a saturated solution of Na_2CO_3 with a pH adjusted to 11.2 using sodium hydroxide, and a 10 mM solution of ammonia solution, which was used for the termination of derivatization process.

Cheese samples

Cheese samples were directly obtained from the manufacturers. For this study, we specifically selected semi-hard and hard ripening cheeses made from goat and sheep milk. The characterization of the samples is presented in Table 1. Samples were collected in duplicates (totally 22 samples were obtained) with an average weight of over 100g per sample. Immediately after collection, the samples were vacuum packed and stored in a freezer at -18°C until further use.

Table 1 Characterization of goat and sheep semi-hard and hard ripening cheeses

Category	Sample identification	Cheese type
Semi-hard and hard ripening goat cheeses	B3.1	Young ripening goat cheese
	B3.2	Semi-hard ripening goat cheese
	B3.3	Hard ripening goat cheese
Semi-hard and hard ripening sheep cheeses	C3.1	Hard ripening sheep cheese
	C3.2	Hard sheep cheese ripening 2 months
	C3.3	Hard sheep cheese ripening 18 months
	C3.4	Hard sheep cheese ripening 3 months
	C3.5	Hard sheep cheese ripening 12 months
	C3.6	Hard sheep cheese ripening 20 months
Steamed cheeses (regardless of the type of milk)	D1.2	Semi-hard steamed sheep cheese, non-smoked
	D1.4	Semi-hard steamed sheep cheese, non-smoked

Sample preparation

The cheese samples were extracted and derivatized using a slightly modified method based on **Komprda et al. (2014)** and **Dadáková et al. (2009)**. Each cheese sample was prepared in duplicate. The whole cheese samples were shredded, and from the shredded material, 2.0 ± 0.01 g samples were directly weighed into 50 ml polypropylene centrifuge tubes. Then, 20 ml of 0.1 M HCl and 0.5 ml of the internal standard solution of 1,7-diaminoheptane were added. The samples were homogenized using a disintegrator and subsequently centrifuged for 15 minutes at 4 °C. The supernatant was then filtered through a KA-1 paper filter (Perštejn, Czech Republic) and a syringe filter PVDF, 0.22 µm, 25 mm (Q-Max, Frisenette, Knebel). One millilitre of the extract was taken for derivatization in amber vials with a total volume of 12 ml. To the sample, 0.5 ml of saturated Na_2CO_3 solution (pH=11.2) was added and mixed on a vortex for 1 minute before adding 1 ml of the derivatization reagent. Afterwards, the vials were placed in a thermostat for 60 minutes, where the derivatization took place at a temperature of 40 °C. The sample was periodically shaken at 15-minute intervals. After the derivatization reaction was completed, the mixture was left to settle for 15 minutes, and then 250 µl of 10 mM ammonia solution was added to remove any unreacted DCl. The mixture was shaken again for 1 minute and left to settle for 30 minutes. The BA derivatives were extracted using 1 ml of diethyl ether. The organic phase was transferred to vials and evaporated under a nitrogen stream. The residue was reconstituted in 0.5 ml of acetonitrile. The samples were then used for HPLC analysis.

HPLC separation

Agilent 1260 Infinity II HPLC instrument (Agilent Technologies, Germany) equipped with DAD detector was used for determination of biogenic amines. The separation was performed using gradient elution of H_2O/ACN (elution time of 25 min: H_2O 35% - 0%; ACN 65% - 100%) on a Zorbax Eclipse XDB C18 column 150 mm × 3.0 mm × 3.5 µm (Agilent Technologies, USA) with an Agilent EC-C18 pre-column 30 mm × 4.6 mm × 2.7 µm (Agilent Technologies, USA) at a flow rate of 0.6 $ml.min^{-1}$. The injection volume for both the standard and samples was 3 µl. The standard mixture and individual samples were injected in triplicates. Quantification data was collected at 254 nm, and the retention time of the standard and UV spectrum were used for the identification of the individual analyzed biogenic amines. The concentration of amines was expressed in $mg.kg^{-1}$ of the original cheese sample.

Data processing

The results of the content of individual amines and total biogenic amine content (sum of tryptamine, 2-phenylethylamine, cadaverine, histamine, tyramine, spermidine, spermine) were expressed in $mg.kg^{-1}$. Data analysis was conducted using Excel software.
Statistical evaluation was performed with use of statistical analysis was carried out in the program Past4.03 (**Hammer *et al*., 2001**). At a significance level of α=0.05, we conducted a correlation analysis between individual biogenic amines. Additionally, principal component analysis (PCA) was performed to assess differences between the samples and sample groups. To examine differences between goat and sheep cheeses, as well as within the sheep cheeses with different ripening periods, ANOVA and Mann-Whitney tests were applied.

RESULTS AND DISCUSSION

The content of biogenic amines in fermented food products can vary significantly due to various factors during processing, including the starter culture, additives, ripening, and maturation. Table 2 provides a summary of the results for individual biogenic amines and the total biogenic amines (the cumulative amount of tryptamine, 2-phenylethylamine, cadaverine, histamine, tyramine, spermidine, and spermine) in hard and semi-hard goat and sheep cheeses.
The results show that histamine was present in 54.5% of the samples, while its average content in the samples ranged from undetectable amounts to 68.7 $mg.kg^{-1}$. We found the highest average content of histamine in sample C3.3 of hard sheep cheese ripening for 18 months (68.7 $mg.kg^{-1}$). In general, histamine was not detected in goat cheese samples, in sheep cheese aged for 20 months and in steamed sheep cheese D1.2. Regarding the histamine distribution in the ripening cheese, **Moniente *et al.* (2022)** stated that histamine has tendency to accumulate in the inner part of cheese whereas the shell part was shown to has the lowest concentrations. The positive effect on histamine development in cheese has higher salt content, higher humidity, and lower oxidation. Tryptamine was found in 63.4% of total samples, however in the goat samples it was detected only in B3.3 sample. Its concentration ranged from non-detectable amounts to 402 $mg.kg^{-1}$. 2-phenyletylamine was observed in 54.5% of samples, where presented the third most abundant representative of biogenic amine. It varied also from non-detectable concentrations to 268 $mg.kg^{-1}$. Cadaverine, with the presence in 72.7% of samples, varied in the samples from non-detectable concentrations to 176 $mg.kg^{-1}$. Tyramine was found in all, but one samples in the concentration range from non-detectable to 381 $mg.kg^{-1}$. Spermidine ranged from non-detectable concentrations to 50.9 $mg.kg^{-1}$ and was present in all goat samples and in 75% of sheep cheeses. Spermine in goat cheeses was relatively similar, but in sheep cheeses ranged from non-

detectable concentrations to 54.0 mg.kg^{-1}. Higher concentrations of biogenic amines are generally found in fermented or ripening cheeses. **Kandasamy *et al.* (2021)** found that total BA levels in farm-fresh cheeses ranged from 11.2 to 62.1 mg.kg^{-1}, while in ripening cheeses they ranged between 257.7 and 384.3 mg.kg^{-1}, which shows a gradual increase during the ripening period. The European Food Safety Authority (EFSA) indicate the maximum content of histamine and tyramine in fresh and hard cheeses, corresponding to 119 and 1240 mg.kg^{-1} and 457 and 1450 mg.kg^{-1}, respectively **(EFSA, 2011)**. The analysed samples in our survey had the contents of both biogenic amines below these permissible values.

In our study, when considering the dominance of individual biogenic amines in cheeses, tyramine and tryptamine were found to be dominant in most of the samples, followed by 2-phenylethylamine and spermine. Spermidine and cadaverine were also present but in lower quantities. Samples of sheep cheeses C3.3 and C3.5 were characterized by high levels of dominant biogenic amines such as tyramine, tryptamine, and 2-phenylethylamine. In comparison to goat cheeses, these samples had a significantly higher concentration of total biogenic amines, approximately 100 times higher. The total biogenic amine content in goat cheese ranged from 15.0 to 91.2 mg.kg^{-1}, while in sheep cheeses, it varied from 14.4 to 1322 mg.kg^{-1}. The total amount of biogenic amines represents the sum of all analyzed amines in the cheese samples.

Table 2 Content of biogenic amines in hard and semi-hard goat and sheep cheeses expressed in mg.kg^{-1} in original material

Sample	Histamine mean±SD	Tryptamine mean±SD	2-PEA mean±SD	Cadaverine mean±SD	Tyramine mean±SD	Spermidine mean±SD	Spermine mean±SD	Total BA mean±SD
B3.1	ND	ND	3.1±0.01	ND	6.8±1.0	1.0±0.01	4.1±0.01	15.0±1.0
B3.2	ND	ND	ND	ND	8.2±0.01	2.1±0.01	6.2±0.01	16.4±0.01
B3.3	ND	44.1±13.0	ND	4.1±0.01	35.9±0.01	1.4±0.5	5.8±0.5	91.2±13.4
C3.1	2.1±0.01	9.2±0.01	8.2±0.01	5.1±0.01	10.6±0.5	2.1±0.01	3.8±0.5	41.0±0.01
C3.2	10.3±0.01	215±17.4	133.6±0.5	103.5±0.01	263.8±0.5	50.9±0.5	54.0±0.5	830.9±16.8
C3.3	68.7±0.8	ND	67.3±0.5	49.9±0.5	974.8±1.7	ND	ND	1160.7±3.4
C3.4	2.1±0.01	46.1±0.8	ND	19.5±0.01	40.0±0.01	5.1±0.01	8.5±0.5	121.3±1.3
C3.5	15.4±0.8	401.5±8.4	267.5±3.0	176.0±61.8	380.6±0.5	50.2±0.01	31.1±0.5	1322.3±51.5
C3.6	ND	38.6±14.0	ND	ND	45.4±0.5	1.0±0.01	ND	85.1±13.8
D1.2	ND	14.4±0.01	8.2±0.01	5.1±0.01	13.3±0.01	3.1±0.01	4.1±0.01	48.2±0.01
D1.4	7.2±0.01	ND	ND	2.1±0.01	ND	ND	5.1±0.01	14.4±0.01
Mean	**9.60**	**69.89**	**44.35**	**33.20**	**161.77**	**10.62**	**11.15**	**340.58**
Median	**2.05**	**14.35**	**3.08**	**5.13**	**35.88**	**2.05**	**5.13**	**85.08**

Legend: SD – standard deviation, ND – not detected, 2-PEA – 2-phenylethylamine

Dicakova *et al.* (2004) reported less than 10 mg.kg^{-1} of biogenic amines in most of the sheep cheeses. In hard and semi-hard cheeses, **O'Callaghan *et al.* (2017)** reported the average histamine content of 100 – 110 mg.kg^{-1} in cheddar and Emmental cheeses, lower concentrations were determined in edam and gouda cheeses (35 mg.kg^{-1}). **Mayer and Fiechter (2018)** published an average histamine content of 204.9 mg.kg^{-1} in hard cheeses and 38.3 mg.kg^{-1} in semi-hard cheeses. **Renes *et al.* (2021)** found the highest representation (more than 90 % of total biogenic amine content) of histamine and tyramine in sheep cheeses, with the exception of summer cheeses after 100 days of ripening, in which the main biogenic amine was cadaverine, followed by tyramine and histamine. These 3 biogenic amines were present in all samples. In sheep ripening Pecorino cheeses, **Schirone *et al.* (2012)** reported significantly variable histamine content ranging from 7.70 to 25.3 mg.kg^{-1} in cheeses aged for 4 months. In cheeses aged for 5 months, the average histamine content was 65.5 mg.kg^{-1}. Goat cheeses were analysed in biogenic amines content by **Novella-Rodríguez *et al.* (2002)**. The authors stated that during ripening the total content of biogenic amines had an increasing tendency. The average content of histamine in goat cheeses from milk processed under high pressure was higher (48.7 ± 19.3 mg.kg^{-1}) than in cheese made from pasteurized milk (23.2 ± 5.10 mg.kg^{-1}). Tyramine, histamine and 2-phenylethylamine, putrescine and cadaverine while the dominant biogenic amines in the cheese. The results of our survey show that the contents of biogenic amines are within the range reported by comparable studies of biogenic amines in ripening hard and semi hard sheep and goat cheeses. The levels of this biogenic amine were not higher than the maximum content stated by the Food Safety Authority (EFSA).

Concentrations of biogenic amines in cheeses were compared using univariate and multivariate statistical tests to assess differences between the cheeses. The Mann-Whitney pairwise test with raw p-values and uncorrected significance was utilized to examine the differences between the cheeses based on the type of milk used. The results of these tests are presented in Table 3.

Table 3 Comparison of individual biogenic amines contents in sheep and goat cheeses

	Goat vs. sheep cheeses	
	p-value	Significance
Tryptamine	0.02446	*
2-phenylethylamine	0.0226	*
Cadaverine	0.0009596	***
Histamine	-	
Tyramine	0.01131	*
Spermidine	0.2499	-
Spermine	0.7278	-
Total content of BAs	0.01583	*

Legend: Statistically significant differences: * p<0.05; ** p<0.01; *** p<0.001

Statistically significant differences were observed in the content of tryptamine, 2-phenylethylamine, cadaverine, tyramine, and the total biogenic amines. However, a comparison for histamine was not feasible as it was undetectable in all goat cheeses. No significant difference was found for spermidine and spermine.

Principal component analysis (PCA) was conducted on three groups of cheese types: ripening goat cheese, ripening sheep cheese, and steamed sheep cheese. This analysis aimed to visualize the differences between these groups (Figure 1) based on the content of individual biogenic amines and the total biogenic amines content. Component 1 represented 69.9% variance and 25.9% was represented by component 2 that means 95.7% total variance. Based on high content of histamine and tyramine, hard sheep cheese ripening 12 months no. C3.3 was shown to be highly different compared to other cheeses. Cheeses C3.2 and C3.5 (hard ripening sheep cheeses) were characterized by high content of cadaverine and comparable levels of spermidine and spermine, as shown in figure 1. Other cheeses are positioned closely to each other on the PCA map due to their similar content of biogenic amines. Based on the variables used in this analysis, it was not possible to distinguish between the different cheese types. It is likely that additional data could improve the differentiation between the cheese types.

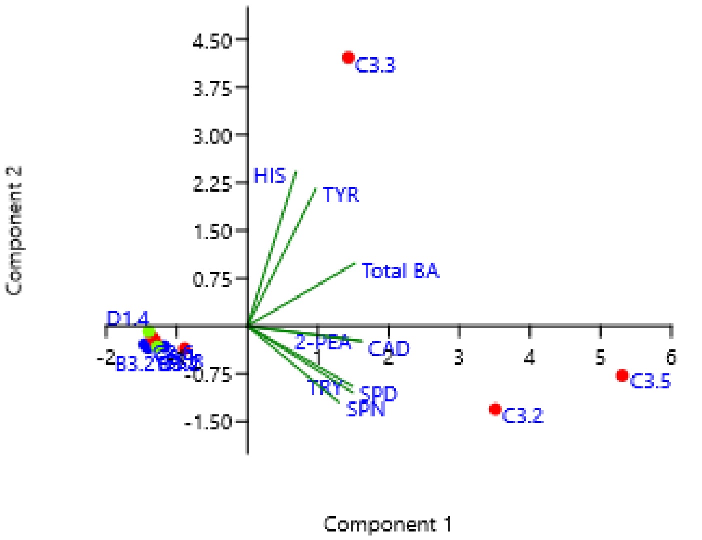

Figure 2 PCA map of sheep and goat cheeses

Note: HIS – histamine, TYR – tyramine, TRY – tryptamine, 2-PEA – 2-phenylethylamine, CAD – cadaverine, SPD – spermidine, SPN – spermine, BA – biogenic amines

The correlation between individual pairs of biogenic amines is depicted in Figure 2. We utilized a model of linear correlation (Pearson) with uncorrected p-values to represent significant correlations (p<0.05). The crossed cells in the figure indicate a significance level of p>0.05, indicating the application of linear correlation statistics (Pearson).

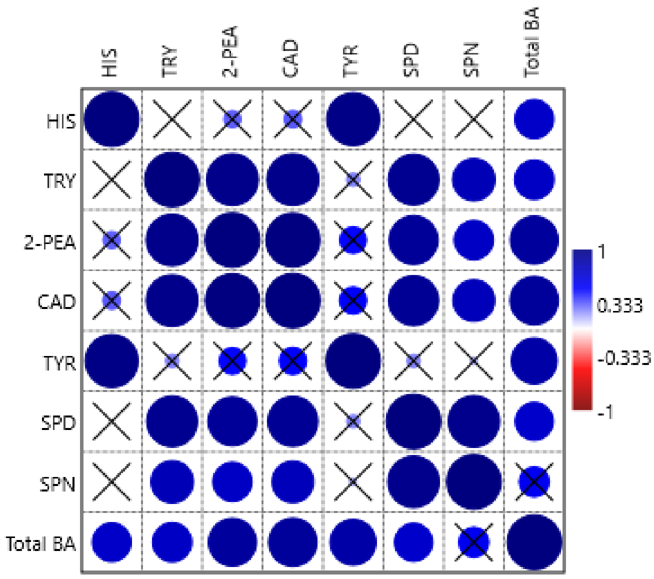

Figure 2 Plot of individual correlations between the biogenic amines in cheeses
Note: Crossed cells – significance level p<0.05, application of linear r (Pearson) correlation statistics with uncorrected p-values; HIS – histamine, TYR – tyramine, TRY – tryptamine, 2-PEA – 2-phenylethylamine, CAD – cadaverine, SPD – spermidine, SPN – spermine, BA – biogenic amines

The total content of biogenic amines exhibited a positive correlation, with the exception of spermine, which showed a lower correlation. A significant correlation was observed between histamine content and tyramine. Tryptamine displayed a high correlation with 2-phenylethylamine, cadaverine, spermidine, and spermine. Additionally, 2-phenylethylamine was found to be highly positively correlated with cadaverine, spermidine, and spermine. Cadaverine also demonstrated a significant correlation with spermidine and spermine. Other relationships showed a low level of positive correlations.
To investigate differences within the content of individual biogenic amines, a dataset of sheep cheeses at different ripening periods was examined. The results of the statistical differences are provided in Table 4.
When comparing the six variants of sheep cheeses with ripening periods ranging from 2 to 20 months, significant differences were observed among all variants for the variables histamine, 2-phenylethylamine, tyramine, spermidine, and spermine. For tryptamine, similarities were found between cheeses with a short ripening period (2 and 3 months) and cheese with the longest ripening period (20 months). In terms of cadaverine content, similarities were particularly observed between cheeses with 3 and 18 months of ripening. However, similar cadaverine contents were also evident in other variants listed in Table 4.
Histamine limits set in the Commission Regulation (EU) No 1019/2013 of 23 October 2013 and Regulation (EC) No 2073/2005 are related only to the fish and fishery products (200 $mg.kg^{-1}$) and fish sauce produced by fermentation of fishery products (400 $mg.kg^{-1}$). Limits for biogenic amines and histamine itself are not set for the other food products. In our study no cheese sample exceeded histamine concentration of 200 $mg.kg^{-1}$. Considering the significance of monitoring biogenic amines in food products that have the potential to contain elevated levels of these compounds, which can adversely affect health, establishing criteria for evaluating the quality of such products poses a challenge. Furthermore, it is important to recognize that certain food items may contain toxic levels of histamine and tyramine without exhibiting noticeable alterations in taste, smell, or appearance. This presents a difficulty for consumers in detecting these harmful levels and avoiding such products. Therefore, it is essential to continuously monitor the presence of biogenic amines in food products to ensure their safety.

Table 4 Comparison of individual biogenic amines contents in sheep cheeses with different ripening period

Ripening time	Histamine	Tryptamine	2-PEA	Cadaverine	Tyramine	Spermidine	Spermine
C3.2 – 2 months	c	bc	b	ab	c	a	a
C3.4 - 3 months	d	c	d	bc	e	b	c
C3.5 - 12 months	b	a	a	a	b	a	b
C3.3 - 18 months	a	d	c	bc	a	d	d
C3.6 - 20 months	e	c	d	c	d	c	d

Legend: in columns representing individual parameters different letters mean a statistically significant difference ($p < 0.05$) between the variants.

CONCLUSION

Biogenic amines are considered risky substances that can occur in higher concentrations in cheeses, emphasizing the importance of monitoring this type of food. The analysis of biogenic amines in hard and semi-hard goat and sheep cheeses involved the extraction of samples with 0.1 M HCl and pre-column derivatization with DCl, followed by analysis using the HPLC-DAD method.
The results of the analysis showed the presence of biogenic amines in the examined samples of hard and semi-hard cheeses. Specifically, histamine content was detected in 54.5% of the samples, and a similar occurrence was observed for 2-phenylethylamine. However, no detectable amount of these amines was found in any of the goat cheese samples. Tryptamine was found in 63.4% of the total samples, cadaverine was present in 72.7% of the samples, and tyramine was detected in all samples except one.

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