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DETERMINATION OF BIOGENIC AMINES IN CULTIVATED AND PROCESSED MUSHROOMS INTENDED FOR THE SLOVAK MARKET

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ABSTRACT

Cultivated and processed white button mushrooms are widely used in the menu of the Slovak consumers. However, these food items can potentially contain biogenic amines, attributed to the presence of precursor compounds and their susceptibility to microbial spoilage. Biogenic amines significantly influence food quality and can pose health risks to consumers. This study is focused on determination of spermidine, putrescine, tyramine, cadaverine, histamine, spermine, and 2-phenylethylamine in processed mushroom products available in the Slovak market. The findings revealed considerable variability in biogenic amine content across individual products, influenced by the producer. Spermidine, putrescine and spermine emerged as the predominant biogenic amines with concentration ranges 2349.8 -7412.8, 37.2 - 607.5 and 74.2 - 267.6 mg/kg DW, respectively. Tyramine and histamine were detected in 22.2% of processed mushroom samples with one product exceeding concentration 200 mg/kg DW. Cadaverine was identified in 44.4% samples. The presence of histamine has to be concerned regarding potential health risks for consumers. Comparison of the mushroom products showed significant differences between the groups of products with whole mushrooms vs. sliced mushrooms in brine in case of the content of spermine ($p<0.001$), water content ($p<0.01$), putrescine, histamine and tyramine ($p<0.05$ for all these BAs). No significant differences were found between the groups for the cadaverine, spermidine and total content of biogenic amines. In house rapid validation was performed in term of determination of limits of detection and limits of quantification and intraday precision of retention time for individual biogenic amines in this study. Limits of detection ranged from 0.013 to 0.029 µg/mL and limits for quantification were in the range from 0.039 to 0.087 µg/mL. The RSD for the retention time in standard solutions did not exceed 0.12%.

Keywords: *Agaricus bisporus*, canned mushrooms, HPLC-DAD, food safety

INTRODUCTION

Biogenic amines (BAs) present a group of commonly occurring compounds with a low molecular weight. Basic biochemical reactions resulting in formation of BAs are decarboxylation of amino acids by microorganisms, reductive amination and transamination of aldehydes and ketones and also by a body tissue activity (**Wójcik et al., 2021**). Thus, BAs may bring an important information on food hygiene and quality and can be evaluated as a potential health threat for consumers due to their physiological and toxicological effects, especially if present in high concentrations (**Jakabová et al., 2023; Ruiz-Capillas, Herrero, 2019; Özogul and Özogul, 2019**). Cultivated fresh and processed mushrooms are widely used ingredients in various dishes in Slovak kitchen. Mushroom products are commonly available on the Slovak market and present a very popular item. Mushrooms offer nutritional advantages as a provider of all essential aminoacids in the form of peptides and proteins (**Yadav & Negi, 2021; González et al., 2020**), non-digestible carbohydrates for dietary fiber, unsaturated fats, minerals, and a variety of vitamins. This has led to an increased intake of mushrooms and the creation of various processed mushroom products (**Yadav & Negi, 2021**). Fresh mushrooms are highly perishable foods, and thus is important technology of their processing to retain important organoleptic and sensory valuable properties, and moreover overcoming the formation of risk components (**Fernandes et al., 2013**). Amino acids identified in mushrooms are threonine, lysine, valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, and recently, histidine. The disadvantage of these amino acids lies in the fact that, due to inappropriate storage, they are susceptible to microbial degradation, which is further facilitated by the neutral pH typical for mushrooms. Metabolites formed during the decomposition process can be toxic to humans, but they also give rise to essential building blocks necessary for the formation of alkaloids, hormones, coenzymes, vitamins, phospholipids, and neurotransmitters themselves. In the decomposition of mushrooms, the biogenic amine cadaverine is also produced, originating from lysine **(Hofrichter, 2018)**. Combination of the presence of the amino acides as substrates for the biochemical processes with appropriate conditions creates the possibility to form biogenic amines. Biogenic amines in human diet are divided into two main subgroups according to different way of their formation and their biological effects (**Dadáková et al., 2009**). The first group is formed by monoamines, depicted by histamine, tyramine, 2-phenylethylamine and tryptamine, then by diamines such as putrescine and cadaverine and triamines represented by agmatine. The second group present polyamines, formed in the living organisms and present the bioactive compounds influencing human health positively, but also negatively, depending on conditions. Originally, spermidine and spermine are classified as polyamines, but their precursor putrescine is also assigned to this group **(Dadáková et al., 2009)**. Mushrooms should be considered as food items that have the potential to contain high levels of biogenic amines (**Jabłońska-Ryś et al., 2022**). As a result of consuming spoiled dishes made from edible mushrooms, secondary poisoning may occur, accompanied by symptoms such as excessive sweating, intestinal colic, a sense of fullness, fever, dizziness, facial redness, warmth, circulatory disorders, chills, and others **(Hofrichter, 2018)**.

Recently, several studies appeared with focus on determination of biogenic amines in mushrooms. Biogenic amines in some mushroom species were investigated by **Reis et al. (2020a)**, **Reis et al. (2020b)**, **Reis et al. (2020c)**, **Dadáková et al. (2009)**, **Dadáková et al. (2022)** resulting in detection of spermidine, agmatine, putrescine, cadaverine, tyramine, tryptamine and 2-phenylethylamine. However, not every mushroom species contained all above-mentioned amines.

Technological processes like sewing, sterilisation, cooking, freezing have impact on final concentration of biogenic amines compared to fresh material (**Dadáková et al., 2022; Jabłońska-Ryś et al., 2020; Jabłońska-Ryś et al., 2022; Bak et al., 2023; Muñoz-Esparza et al., 2021)**. Thus, the understanding of the processes can help to improve the quality and safety of food products.

The objective of this work was to evaluate profile and levels of eight commonly present biogenic amines in the processed edible mushrooms available in the Slovak market in context to consumer safety.

MATERIAL AND METHODS

Reagents and Standard Solutions

Putrescine, cadaverine and histamine in the form of dihydrochloride (≥98%), 2-phenylethylamine (≥98%), tyramine and tryptamine hydrochloride (≥99%), spermidine trihydrochloride (≥99%), spermine tetrahydrochloride (≥99%), and

1,7-diaminoheptane (≥99%) were obtained from Merck KGaA (Darmstadt, Germany), as well as dansylchloride (DCL, ≥99%). Centralchem (Bratislava, Slovakia) provided us with a hydrochloric acid (35%, p.a.), sodium carbonate (p.a.) and ammonia (26%, p.a.). The ultrapure water was prepared in Milli-Q water purification system (Millipore, France). The solvents 2-propanone (≥99%), diethylether (≥99%) and acetonitrile (≥99.9%) (Sigma-Aldrich, Darmstadt, Germany) were of HPLC quality.
Standard mixture was prepared by mixing of 40 mg of each standard with water in 100 ml volumetric flask from amber glass. Amount of 1 mL of the standard mixture was derivatized and reconstructed in acetonitrile and introduced to the HPLC system for preparation of calibration curve by injecting of different amounts of the standard mixture. Derivatization process was optimized and described in the work of **Jakabová et al. (2023)**.

Mushroom samples

Mushroom samples (*Agaricus bisporus*) were purchased in the Slovak supermarkets in the form of canned sterilized products, packed in cans or glass jars. The weight of the product ranged from 184 to 400 g with the solid content between 100 and 230 g. Two common forms of the canned button mushrooms were used in this study – whole and sliced. Table 1 shows the characterisation of the samples. Immediately after opening the cans or jars, solid content – whole mushrooms were sliced to smaller pieces, let to drip on the filter paper, then homogenized in hand blender (Bosch MSM67160 ErgoMixx, Robert Bosch GmbH, Gerlingen, Germany), and lyophilized in the freeze dryer Telstar Lyoquest-55 (Azbil Telstar Technologies SLU, Barcelona, Spain). The content of water was determined in freeze dried material and this data was used for a dry weight in expressing the results. Consequently, freeze dried samples were homogenized to powder in mortar with pestle and 1.00± 0.01g was weighed directly into 50 mL plastic (PP) tubes for centrifugation. In the first step, the samples were disintegrated with use of disintegrator SilentCrusher M (Heidolph Instruments GmbH, Schwabach, Germany) with 25 mL of HCl (c=0.1mol/L) and then centrifugation was applied for 10 min at relative centrifugal force 3420. Supernatant was taken in amount of 1 mL and put into glass vials with a total volume of 12 mL.

Table 1 Characterization of processed mushroom products (data from producers)

Sample identification	Product specification	Mushroom Form	Net weight (g)	Drained weight (g)	Type of package	Country of origin
SK_01	button mushrooms in brine	whole	280	170	glass jar	Not given
SK_03	button mushrooms in brine	whole	400	230	can	Netherlands
SK_04	button mushrooms in brine, sterilized	sliced	280	156	can	Netherlands
SK_05	button mushrooms in brine, sterilized	sliced	290	155	can	Netherlands
SK_06	button mushrooms in brine	sliced	200	115	can	Poland
SK_07	button mushrooms in brine	whole	290	156	can	Not given
SK_08	button mushrooms in brine, sterilized	sliced	400	210	can	Netherlands
SK_09	button mushrooms in brine	sliced	290	156	can	Not given
SK_10	button mushrooms in brine	sliced	184	100	can	Not given

Sample preparation

The derivatization was done according to slightly modified methods of **Komprda et al. (2014)** and **Dadáková et al. (2009)**. Saturated solution of sodium carbonate with pH adjusted to 11.2 was added in amount of 0.5 mL and vortex mixed for 1 minute. Derivatization agent, 2-propanone solution of DCL (c=5 mg/mL), was added in amount of 1 ml to supernatant and kept in dark in drying and heating chamber WTC Binder (Binder GmbH, Tuttlingen, Germany) at 40°C for 60 min with 15-minute intervals of shaking. After cooling of derivatized samples, ammonia solution (250 µl, c=10 mmol/L) was pipetted and vortexed to stop derivatization process. BAs were extracted to diethyl ether (added in liquid-liquid extraction in amount of 2 mL), which was transferred to HPLC amber vials and evaporated in nitrogen mini evaporator. Samples were reconstructed with 0.5 ml of acetonitrile and dissolved with use of vortex for 1 min in Heidolph Reax top vortex (Heidolph Instruments GmbH, Schwabach, Germany), followed by the HPLC analysis.

Determination of BAs

The analysis of biogenic amines was conducted using the Agilent 1260 Infinity II HPLC instrument (Agilent Technologies, Santa Clara, CA,
United States) equipped with a DAD detector. The separation process involved a gradient elution of H_2O/ACN over 25 minutes (elution profile: H_2O 35% - 0%; ACN 65% - 100%) on a Zorbax Eclipse XDB-C18 column sized at 150 mm × 3.0 mm × 3.5 µm (Agilent Technologies, USA), along with an Agilent EC-C18 pre-column sized at 30 mm × 4.6 mm × 2.7 µm (Agilent Technologies, USA). The flow rate was maintained at 0.6 mL/min, and the injection volume for both standard and sample solutions was 3 µL. Triplicate injections were done for both the standard mixture and individual samples. Data acquisition was performed at 254 nm, and the retention time, and the UV spectra were used for the identification of the analytes. The concentration of BAs was reported in mg/kg on a dry weight basis of the sample.

Data analysis

The outcomes regarding the concentrations of specific amines and the overall biogenic amine content (comprising tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine) were expressed in mg/kg DW. Individual biogenic amines were tested for determination of limit of detection (LOD) and limit of quantification (LOQ) based on the calibration curves, constructed from 5 calibration concentration levels of standard mixtures of BAs, which were measured in five repetitions per one concentration level of standard mixture of BAs. Calibration solutions of standard mixtures of BAs were prepared with equidistant distribution in the concentration range of individual BAs 0.05 – 0.25 µg/mL. Before expression of results of individual BAs concentrations in mushroom products, evaluation of results was performed from the point of comparison with LOD and LOQ in solutions. Data analysis was carried out utilizing Excel software for descriptive statistics and for preparation of percentage distribution of BAs in mushroom products. For statistical assessment, the program Past4.03 (**Hammer et al., 2001**) was employed. Data normality was tested with a normality test and based on the results, investigation of variations between sliced and whole button mushrooms, ANOVA and Mann-Whitney tests were applied. The results of the normality test showed that normal distribution was in case of water content and other parameters did not have normal distribution, thus the Mann-Whitney test was used for these parameters. At a significance level of α=0.05, relationships between variables were tested using correlation analysis. Furthermore, principal component analysis (PCA) was conducted to evaluate distinctions among the groups of whole and sliced mushroom products.

RESULTS AND DISCUSSION

Selected validation parameters including LOD, LOQ and intraday precision of retention time were calculated, based on individual calibration levels of standard mixture of biogenic amines, are shown in the Table 2. LOD values for individual BAs were in the range 0.013– 0.029 µg/mL and LOQ values ranged from 0.039 to 0.087 µg/mL. Intraday RSD of retention time in standard solutions was between 0.01% (spermine) and 0.12% (histamine).

Table 2 Selected validation parameters for individual biogenic amines

Parameter	TRM	2-PEA	PUT	CAD	HIM	TYM	SPD	SPM
LOD (µg/mL)	0.018	0.025	0.029	0.019	0.020	0.029	0.013	0.016
LOQ (µg/mL)	0.053	0.075	0.087	0.056	0.061	0.087	0.039	0.048
Retention time (min., mean±SD)	3.479±0.003	4.644±0.004	5.304±0.005	6.216±0.007	6.885±0.008	11.348±0.003	12.425±0.004	15.603±0.002

Note: TRM – tryptamine; 2-PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIM – histamine; TYM – tyramine; SPD – spermidine; SPM – spermine; LOD – limit of detection; LOQ – limit of quantification; SD – standard deviation of retention time.

Table 3 provides a summary of the observations for analytes including individual biogenic amines and the total BAs (as a sum of amounts of individual BAs) in white button mushrooms. Based on the summary statistics and percentage distribution of BAs in mushroom products (Table 3, Figure 1), putrescine, spermidine and spermine were present in all samples, cadaverine in 44.4% of samples, histamine, and tyramine in 22.2% samples and tryptamine and 2-phenyletylamine were not observed in the samples. From this point of view, we do not show 2-phenylethylamine and tryptamine in the tables. Normal probability test showed normal distribution for the water content in the samples, other variables had correlation coefficients lower than 0.95.

Table 3 Content of water in % and biogenic amines in processed white button mushrooms expressed in mg/kg in DW, and summary statistical description of data

Sample	Water content %	Putrescine mean±SD	Cadaverine mean±SD	Histamine mean±SD	Tyramine mean±SD	Spermidine mean±SD	Spermine mean±SD	Σ BAs mean±SD
SK_01	93.1	109.3±3.1	265.3±6.6	222.1±1.0	70.4±0.9	3675.2±83.0	85.6±2.0	4427.9±82.4
SK_03	94.5	110.1±1.6	ND	ND	ND	2802.2±16.7	88.3±0.7	3000.7±18.8
SK_04	94.2	125.8±0.2	ND	ND	ND	3224.0±6.4	117.3±0.2	3467.1±6.4
SK_05	94.4	104.3±0.8	ND	ND	ND	2490.9±3.9	105.5±0.2	2700.8±4.3
SK_06	94.7	103.1±0.2	ND	ND	ND	3280.4±8.2	111.7±0.5	3495.2±8.9
SK_07	94.2	38.1±0.8	ND	ND	ND	2354.2±3.1	74.5±0.2	2466.8±4.0
SK_08	93.1	227.1±0.2	30.0±1.3	ND	ND	3778.2±4.6	113.4±0.2	4148.7±5.0
SK_09	95.1	169.9±2.6	62.5±1.1	ND	44.3±1.3	3328.0±3.6	190.2±0.2	3795.0±8.0
SK_10	95.1	606.8±0.6	138.4±0.6	129.2±0.7	ND	7403.9±7.1	267.5±0.2	8545.8±7.9
Summary statistical description								
%	100.0	100.0	44.4	22.2	22.2	100.0	100.0	100.0
Min	**93.1**	**37.2**	**28.7**	**128.3**	**42.6**	**2349.8**	**74.2**	**2461.2**
Max	**95.1**	**607.5**	**271.8**	**223.5**	**71.5**	**7412.8**	**267.6**	**8555.4**
Mean	**94.3**	**177.2**	**124.1**	**175.7**	**57.4**	**3593.0**	**128.2**	**4005.3**
SD*	**0.7**	**162.6**	**94.6**	**50.9**	**14.3**	**1451.1**	**59.5**	**1748.5**

Note: SD – standard deviation for individual analytes in the samples – precision of the analytical method, SD* – standard deviation in summary statistical evaluation of all products analyzed, ND – not detected, BAs – biogenic amines, % – percentage of positive findings within all investigated products

The content of biogenic amines in processed mushrooms expressed in mean values and standard deviation is listed in Table 3. The content of biogenic amines in processed food products can vary significantly due to various factors during processing or different culinary practices. **Muñoz-Esparza et al. (2021)** reported the effect of domestic cooking processes on polyamine levels. Based on their investigations, polyamine levels in food decrease by up to 64% by boiling and grilling, however microwave and sous-vide cooking did not have significant effect. *A. bisporus*, either fresh or processed (including cooking and canning), is documented a rich source of polyamines (**Reis et al., 2020a)**. Spermidine, as a representative of polyamines, emerged as the predominant biogenic amine identified in the mushroom samples (Table 2). In the processed samples of white button mushrooms, the most popular and widely consumed mushrooms in Slovakia, the spermidine content varied from 2349.8 to 7412.8 mg/kg DW. These results are in agreement with findings of **Jabłońska-Ryś et al. (2020)**, **Reis et al. (2020b),** and **Reis et al. (2020c),** and who reported the highest abundancy of spermidine in unprocessed and processed white button mushrooms. Canning was identified to cause higher decrease of spermidine compared to fresh and cooked mushrooms (**Reis et al., 2020a)**. The presence of spermidine in mushrooms, as well as in all living cells, has been documented in the literature for wild mushrooms (**Dadáková et al., 2009; Kalač, 2013**). Its occurrence is linked to various significant functions in cellular metabolism and growth, and it is inherent to mushrooms **(Reis et al., 2020b)**.

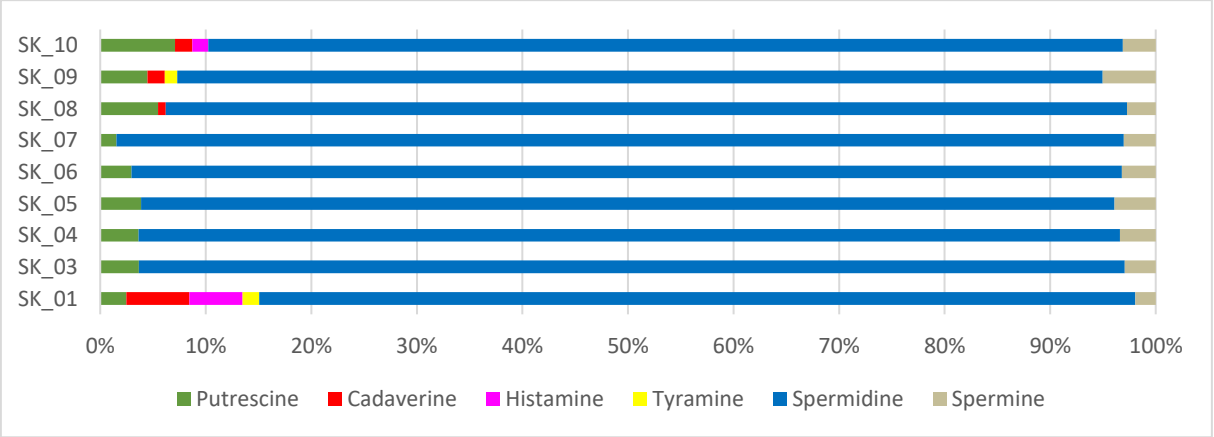


Figure 1 Percentage distribution of BAs in mushroom products

Spermine, classified also as a polyamine, was found in all investigated mushroom products in the range between 74.2 and 267.6 mg/kg DW. **Yang et al. (2020)** investigated polyamines in *A. bisporus* during the heat treatment and storage and spermine content showed more or less constant concentration at a very low level (0.42–1.11 mmol/kg FW). In our experiment spermine was observed as a third most abundant biogenic amine compared to the rest of investigated amines.

As a second the most abundant biogenic amine was putrescine with the highest concentration 606.8±0.6 mg/kg DW, but in most of the samples was present within the range 100–170 mg/kg DW. **Jabłońska-Ryś et al. (2022)** reported in their study content of putrescine in the fermented mushrooms in the range from 0.58 ± 0.25 to 10.11 ± 0.5 mg/kg, that is significantly lower content than in our study. The role of the putrescine is as precursor of polyamines (**Reis et al., 2020b)**, and in some mushroom species is not present in high levels. The high level of putrescine is linked with the transformation of amino acids during microbial degradation and may relate to the insufficient freshness of raw material **(Kalač, 2013; Hofrichter, 2018)**. **Bartkiene et al. (2023)** reported that fermented products including mushrooms are expected to contain low quantities of BAs.

Similar role and origin as putrescine has cadaverine, which was present from non-detectable amounts in 44.4% of samples to the highest concentration in samples SK_01 and SK_10 in amounts 271.8 and 138.4 mg/kg DW, respectively. **Jabłońska-Ryś et al. (2020)** reported cadaverine in the pickled mushrooms in concentration of 259.3 mg/kg, that fits to our results. **EFSA Panel on Biological Hazards (2011)** have not proposed limits for putrescine and cadaverine, however

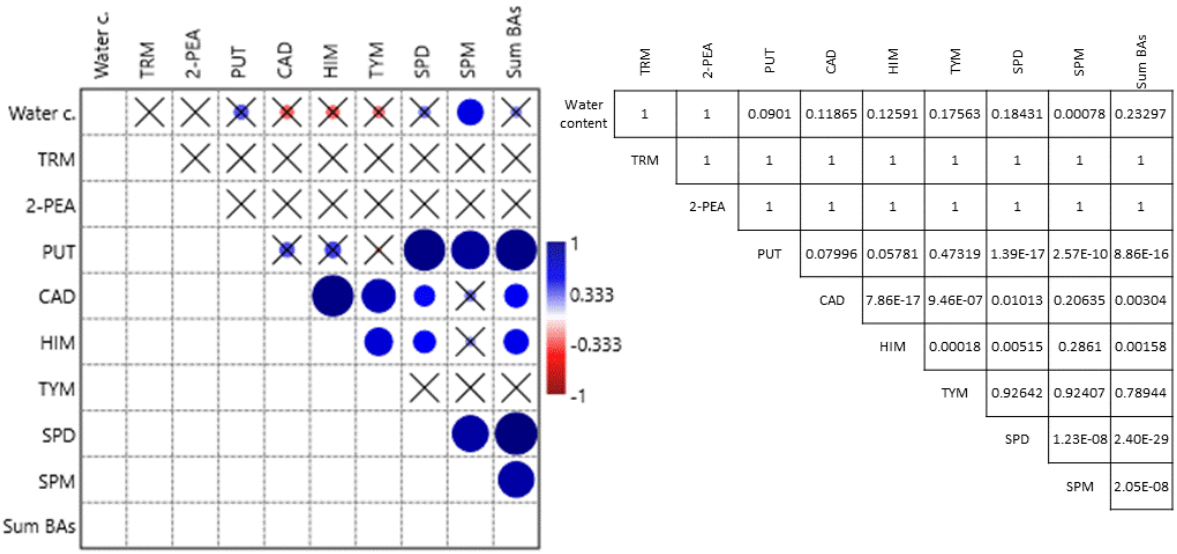
putrescine was reported to have acute oral toxicity on rats at the level 2000 mg/kg body weight **(Til et al., 1997)**.

Tyramine was observed in two products on the concentration levels 44.3±1.3 mg/kg DW and 70.4±0.9 mg/kg DW, respectively. These data are significantly higher than in previously published works of **Reis et al. (2020)**, but similar results have been found by **Jabłońska-Ryś et al. (2022)** in fermented mushroom products. Tyramine was not detected at all in most of the products that agrees with previously published studies (**Kalač, 2013; Jabłońska-Ryś et al., 2020**).

Histamine was present in two products SK_01 and SK_10 with concentration 129.2±0.7 and 222.1±1.0 mg/kg DW and in other products was not detected. As **El-Kosi et al. (2009)** indicated, the levels of these BAs in food items should not exceed the range of 50 to 100 mg/kg for histamine and 100 to 800 mg/kg for tyramine. Histamine and tyramine are considered as the most detrimental BAs, however only histamine has the legal limits, which are set for the fish and fish products and cannot exceed concentrations in the products over 100 and 200 mg/kg **(Commission Regulation EU, 2013)**.

Evaluation of total biogenic amines as a sum of individual BAs determined in individual products showed high variability within the range from 2466.8 (in the product SK_07) to 8545.8 mg/kg DW (in the product SK_10). Overall evaluation of abundancy and content of individual BAs showed that the products vary especially based on the content of three most abundant BAs - spermidine, spermine and putrescine.

The correlation between the individual pairs of BAs is presented in Figure 2. The model used is linear correlation (Pearson) with uncorrected p-values to represent correlations on the significance level p<0.05. The crossed cells in the figure indicate insignificance of correlation.



	TRM	2-PEA	PUT	CAD	HIM	TYM	SPD	SPM	Sum BAs
Water content	1	1	0.0901	0.11865	0.12591	0.17563	0.18431	0.00078	0.23297
TRM	1	1	1	1	1	1	1	1	1
2-PEA	1	1	1	1	1	1	1	1	1
PUT	0.07996	0.05781	0.47319	1.39E-17	2.57E-10	8.86E-16			
CAD	7.86E-17	9.46E-07	0.01013	0.20635	0.00304				
HIM	0.00018	0.00515	0.2861	0.00158					
TYM	0.92642	0.92407	0.78944						
SPD	1.23E-08	2.40E-29							
SPM	2.05E-08								

Figure 2 Correlations between individual analytes in mushroom products

Note: Significance level p<0.05; application of linear r (Pearson) correlation with uncorrected p-values (individual p-values are shown on right side); Water c. – water content; TRM – tryptamine; 2-PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIM – histamine; TYM – tyramine; SPD – spermidine; SPM – spermine; Sum BAs – total content of biogenic amines

Water content showed only significant positive correlation (p=0.0008) with the content of spermine. Strong positive correlations were observed between the polyamines (spermidine and spermine) with putrescine (p<0.001) that logically confirm the origin of these polyamines in the term of putrescine being the precursor of these polyamines in the metabolic pathways. The polyamines correlated with the total BAs due to their dominant contribution to the total BAs variable. A significant positive correlation was observed between histamine and cadaverine content, as was also observed in our previously published work **Jakabová et al. (2023)**. Tyramine displayed a high correlation (p<0.001) with cadaverine and histamine. Other relationships were insignificant.

The levels of biogenic amines in white button mushrooms were analysed through both univariate and multivariate statistical tests to evaluate variations among the two types of the products based on the different sizes of mushroom material. The Mann-Whitney pairwise tests, employing raw p-values and uncorrected significance, was applied to assess differences in the mushroom products containing whole mushrooms and sliced mushroom material. Table 4 showed the p-values calculated and corresponding significance.

Table 4 Comparison of the water content and individual BAs in whole vs. sliced mushrooms in the products

	Whole (n=3) vs. sliced (n=6) mushrooms products	
	p-value	Significance
Water content	0.0091	**
Putrescine	0.0155	*
Cadaverine	0.2623	-
Histamine	0.0338	*
Tyramine	0.0338	*
Spermidine	0.3977	-
Spermine	0.0004	***
Total content of BAs	0.6201	-

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

Statistically significant difference on the level p<0.001was observed in case of spermine between the compared groups. Water content was observed to be significantly different in both groups of whole and sliced-mushroom material on the significance level p<0.01. Significant differences with p<0.05 were observed in the content of putrescine, histamine and tyramine. In case of cadaverine, spermidine and total content of BAs, no significant differences were found between the groups.

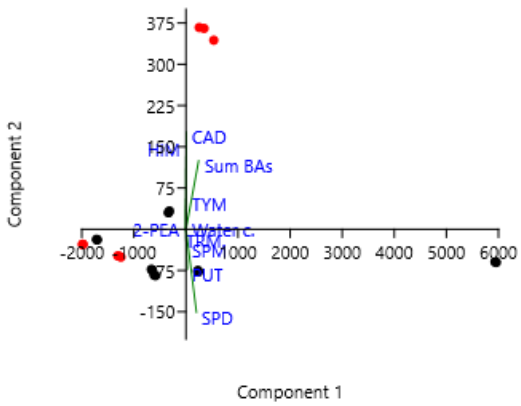


Figure 3 PCA map of white button mushroom products

Note: Component 1 – x-axis of PCA biplot, Component 2 – y-axis of PCA biplot, W – water content; TRY – tryptamine; 2-PEA – 2-phenylethylamine; PUT – putrescine; CAD – cadaverine; HIS – histamine; TYR – tyramine; SPD – spermidine; SPN – spermine; Sum BAs – total content of biogenic amines; red dots – products with whole mushrooms; black dots – products with slices of mushrooms.

As a multidimensional data analysis, a principal component analysis (PCA) was used. Two groups were tested, differing in form of mushrooms (whole white button mushrooms in brine and sliced white button mushrooms in brine). The plot of the analysis is shown in figure 3. The PCA describes the variation and account for the varied influences of the original individual concentrations of biogenic amines and water content. Component 1 explains 99.6% variance and 0.3% explains the component 2, that together presents 99.9% of total variance. Component 1 is characterized especially by high values of loading coefficients (in the range 0.6 – 0.8) for spermidine and sum of BAs and component 2 is represented especially by the loading coefficients for cadaverine, histamine, tyramine between 0.15 and 0.6). Based on high content of cadaverine, histamine and total BAs, sample no. SK_01

very differ from the other products. Other whole mushroom product SK_07 was characterized by the higher content of putrescine and mean contents of spermidine and total BAs. In the group of sliced button mushroom products, SK_10 was outlier product, characterized by the highest content of putrescine and spermidine and presence of cadaverine and histamine, compared to the rest of the products from this group. Similar properties had the rest of the sliced-mushroom products group that resulted to the similar position of the dots in the PCA plot. Position of other mushroom products is close to each other on the PCA map due to their similar composition of BAs.

Limits of histamine content in food is regulated by the **Commission Regulation (EU) No 1019/2013** of 23 October 2013 and **Commission Regulation (EC) No 2073/2005** of 15 November 2005**,** and the limits are related only to the fish and fishery products (200 mg/kg) and fermented fish products (400 mg/kg). Limits for biogenic amines and histamine alone are not set for the other food products, however some recommendation for safe doses in food (per healthy person per meal) were reported as follows: 50 mg histamine and 600 mg tyramine **(EFSA, 2011)**. In our study one product exceeded histamine concentration of 200 mg/kg in DW, but it is necessary to mention, that processing and cooking practices contribute to the decrease of the BAs content, due to its use in original form and dilution by mixing the product with other ingredients in final meal. The presence of histamine, for example, is notable as it is associated with allergic reactions. Monitoring and understanding the levels of histamine and other biogenic amines in mushrooms are essential for assessing potential health risks, especially for individuals with sensitivity or allergies.

Given the importance of monitoring biogenic amines in food products that may harbour elevated levels with potential health implications, establishing criteria for assessing the quality of such products becomes a challenging task. It is important to consider the fact that specific food products could contain high concentrations of histamine and tyramine without displaying noticeable changes in taste, smell, or appearance. This complicates the ability of consumers to detect harmful levels and avoid such products. Hence, there is a continuous need to monitor the presence of biogenic amines in food products to ensure their safety.

CONCLUSION

The analysis of biogenic amines in mushrooms is a complex field that requires a comprehensive understanding of various factors influencing their presence. The presence of biogenic amines in canned button mushrooms can depend on several factors, including the initial composition of the mushrooms, the processing methods including canning, and the storage conditions. The investigation of biogenic amines in product of white button mushrooms was performed in mushroom extracts with DCL pre-column derivatization, followed by analysis by the HPLC-DAD method. The results of the analysis showed the presence of biogenic amines in the examined samples of mushroom products. Specifically, polyamines like spermidine, spermine ant their precursor putrescine were detected in all examined products. Cadaverine was found in four products. Histamine and tyramine were detected in two products, and in one product on the level over 200 mg/kg DW for histamine. Topic of biogenic amines is complex, and mushrooms are considered as a relatively rich for the biogenic amines, especially spermidine and spermine. However, in this study of canned white button mushrooms, only histamine and tyramine were found in elevated levels in some products, but considering the serving of the products in meals, intake of histamine and tyramine cannot reach in common mushroom meals such doses that could cause serious problems to healthy individuals. In general, we can conclude that canned white button mushrooms are safe for consumption from the point of view of original product. Despite of that, mushrooms and mushroom products were identified as sources of BAs, thus monitoring this category of food is important.

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REFERENCES

Bak, K. H., Bauer, S., Rattner, J., Wagner, M., & Ludewig, M. (2023). Nutritional properties, microbial and sensory quality, and formation of biogenic amines in wild-grown mushrooms (*Cantharellus Cibarius* & *Boletus Edulis*) from Austrian local markets. *Food Chemistry Advances*, 2, 100193. https://doi.org/10.1016/j.focha.2023.100193

Bartkiene, E., Zarovaite, P., Starkute, V., Mockus, E., Zokaityte, E., Zokaityte, G., Rocha, J.M., Ruibys, R. & Klupsaite, D. (2023). Changes in lacto-fermented *Agaricus bisporus* (white and brown varieties) mushroom characteristics, including biogenic amine and volatile compound formation. *Foods*, 12(13), 2441. https://doi.org/10.3390/foods12132441

Commission Regulation (EU). (2013). No 1019/2013 of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products. *Official Journal*, 282, 46-47.

Commission Regulation (EC). (2005). No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

Dadáková, E., Pelikánová, T., & Kalač, P. (2009). Content of biogenic amines and polyamines in some species of European wild-growing edible mushrooms. *European Food Research and Technology*, 230, 163-171. https://doi.org/10.1007/s00217-009-1148-3

Dadáková, E., Pelikánová, T., Wolfová, P., & Kalač, P. (2022). Can biogenic amines cause ailments following the intake of edible mushroom meals?. *Czech Journal of Food Sciences*, 40(6). https://doi.org/10.17221/96/2021-CJFS

EFSA Panel on Biological Hazards (BIOHAZ). (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal*, 9(10), 2393. https://doi.org/10.2903/j.efsa.2011.2393

El-Kosi, O. H. R., Abdel-Hakiem, E. H., Ayesh, A. M., & Mohamed, J. I. I. (2009). Effect of different storage temperatures and periods on biogenic amines formation in Ras cheese. *Suez Canal Veterinary Medical Journal*, 1, 207-219.

Fernandes, Â., Barros, L., Barreira, J. C., Antonio, A. L., Oliveira, M. B. P., Martins, A., & Ferreira, I. C. (2013). Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. *LWT-Food Science and Technology*, 54(2), 493-499. https://doi.org/10.1016/j.lwt.2013.06.027

González, A., Cruz, M., Losoya, C., Nobre, C., Loredo, A., Rodríguez, R., Contreras, J., & Belmares, R. (2020). Edible mushrooms as a novel protein source for functional foods. *Food & Function*, 11(9), 7400-7414. https://doi.org/10.1039/d0fo01746a

Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 2001, 4, 9.

Hofrichter, R., (2018). Tajný život húb. Ikar a.s., pp. 232, ISBN 978-80-265-0771-0

Jabłońska-Ryś, E., Sławińska, A., Skrzypczak, K., Kowalczyk, D., & Stadnik, J. (2022). Content of biogenic amines and physical properties of lacto-fermented button mushrooms. *Applied Sciences*, 12(18), 8957. https://doi.org/10.3390/app12188957

Jabłońska-Ryś, E., Sławińska, A., Stachniuk, A., & Stadnik, J. (2020). Determination of biogenic amines in processed and unprocessed mushrooms from the Polish market. *Journal of Food Composition and Analysis*, 92, 103492. https://doi.org/10.1016/j.jfca.2020.103492

Jakabová, S., Árvay, J., Benešová, L., Zajác, P., Čapla, J., Čurlej, J., & Golian, J. (2023). Evaluation of biogenic amines in goat and sheep cheeses of Slovak origin. *Journal of Microbiology, Biotechnology & Food Sciences*, 13(2). https://doi.org/10.55251/jmbfs.10000

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food chemistry*, 113(1), 9-16. https://doi.org/10.1016/j.foodchem.2008.07.077

Komprda, T., Dohnal, V., Cwiková, O. (2014). Chromatografické stanovení biogenních aminů a polyaminů ve zrajících sýrech. *Chemické listy*, 108(12), 1140-1144.

Muñoz-Esparza, N. C., Costa-Catala, J., Comas-Basté, O., Toro-Funes, N., Latorre-Moratalla, M. L., Veciana-Nogués, M. T., & Vidal-Carou, M. C. (2021). Occurrence of polyamines in foods and the influence of cooking processes. *Foods*, 10(8), 1752. https://doi.org/10.3390/foods10081752

Özogul, Y., Özogul, F. (2019). Biogenic amines formation, toxicity, regulations in food. In *Biogenic Amines in Food: Analysis, Occurrence and Toxicity*, (Eds. Saad, B., Tofalo, R.) p. 329, ISBN: 978-1-78801-581-3, https://doi.org/10.1039/9781788015813

Reis, G. C., Guidi, L. R., Fernandes, C., Godoy, H. T., & Gloria, M. B. A. (2020a). UPLC-UV method for the quantification of free amino acids, bioactive amines, and ammonia in fresh, cooked, and canned mushrooms. *Food Analytical Methods*, 13, 1613-1626. https://doi.org/10.1007/s12161-020-01777-5

Reis, G. C., Custódio, F. B., Botelho, B. G., Guidi, L. R., & Gloria, M. B. A. (2020b). Investigation of biologically active amines in some selected edible mushrooms. *Journal of Food Composition and Analysis*, 86, 103375. https://doi.org/10.1016/j.jfca.2019.103375

Reis, G. C., Dala-Paula, B. M., Tavano, O. L., Guidi, L. R., Godoy, H. T., & Gloria, M. B. A. (2020c). In vitro digestion of spermidine and amino acids in fresh and processed *Agaricus bisporus* mushroom. *Food Research International*, 137, 109616. https://doi.org/10.1016/j.foodres.2020.109616

Ruiz-Capillas, C., Herrero, A. M. (2019). Impact of biogenic amines on food quality and safety. *Foods*, 8(2), 62. https://doi.org/10.3390/foods8020062

Til, H. P., Falke, H. E., Prinsen, M. K., & Willems, M. I. (1997). Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. Food and Chemical Toxicology, 35(3-4), 337-348. https://doi.org/10.1016/S0278-6915(97)00121-X

Wójcik, W., Łukasiewicz, M., & Puppel, K. (2021). Biogenic amines: Formation, action and toxicity – A review. *Journal of the Science of Food and Agriculture*, 101(7), 2634-2640. https://doi.org/10.1002/jsfa.10928

Yadav, D., & Negi, P. S. (2021). Bioactive components of mushrooms: processing effects and health benefits. *Food Research International*, 148, 110599. https://doi.org/10.1016/j.foodres.2021.110599

Yang, K. X., Xi, Z. A., Zhang, Y. X., Sheng, J. P., & Meng, D. M. (2020). Polyamine biosynthesis and distribution in different tissues of Agaricus bisporus during postharvest storage. *Scientia Horticulturae*, 270, 109457. https://doi.org/10.1016/j.scienta.2020.109457