

## AMINO ACID PROFILE AND EFFECT OF BAKING AND FROZEN STORAGE ON THE FORMATION OF BIOGENIC AMINES IN THE MEAT OF COMMON CARP (*CYPRINUS CARPIO*)

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### ABSTRACT

This study examines the amino acid composition and biogenic amine formation in common carp (*Cyprinus carpio*) meat subjected to baking and frozen storage for one and three months. Amino acids were analyzed using cation-exchanged liquid chromatography in hydrolysates, while biogenic amines were determined via reverse-phase high-performance liquid chromatography with pre-column derivatization using dansyl chloride. The analysis focused on 15 amino acids, with glutamic acid being the most abundant and tyrosine the least present in both fresh and baked samples. The total amino acid content decreased from 364.5 g/kg DW in fresh carp meat to 314.2 g/kg DW in baked samples.

Biogenic amines, organic compounds formed through amino acid breakdown, were also evaluated due to their implications for food quality and consumer health. Four biogenic amines—2-phenylethylamine, putrescine, spermidine, and spermine—were detected. Their levels increased in the following order: fresh meat < frozen meat (1 month) < frozen meat (3 months) < baked meat. Initial concentrations of putrescine (2.80 mg/kg DW) and spermidine (15.23 mg/kg DW) in fresh carp meat rose significantly after freezing and baking, with spermine also detected after one month of storage. Tyramine levels increased significantly in baked samples. Statistical analysis using Tukey's test showed significant differences ( $p < 0.05$ ) among the variables under described experimental conditions, indicating the need for further studies to monitor biogenic amine trends in processed freshwater fish products.

**Keywords:** common carp, amino acids, biogenic amines, frozen storage, baking

### INTRODUCTION

Biogenic amines are organic compounds that receive considerable attention in nutrition. Low concentrations of biogenic amines are naturally found in many foods, where they occur as natural metabolic products or intermediates. They are most commonly formed through the relevant decarboxylation of amino acids in enzymatic reactions of tissues or microbial processes (Křížek *et al.*, 2002; Velíšek, 2002). Higher concentrations of biogenic amines can be found in fermented foods such as cheeses, cured sausages, beer, wine, sauerkraut, etc. (Velíšek, 2002). Biogenic amines are formed especially in bacteria assisted decarboxylation of free amino acids in food (Li *et al.*, 2018; Wójcik, *et al.*, 2021). Histidine, lysine, and arginine are precursors for the formation of histamine, cadaverine, and putrescine (Buňka *et al.*, 2013). In some non-fermented foods, the presence and content of biogenic amines primarily indicate the quality of the original material/food and the standard of the hygiene chain, especially during storage (Bita, Sharifian, 2024). This is particularly true for fish and fish products (Křížek *et al.*, 2002). The types and levels of biogenic amines present in various fish species can differ significantly. The presence of substrate amino acids is essential for the synthesis of biogenic amines (BAs). Proteolysis plays a key role in this process, as it directly influences the availability of free amino acids that serve as substrates for BAs production (EFSA, 2012). Other key internal factors influencing biogenic amines content in fish meat, similar to other types of meat, include the pH, ionic strength, nutrient composition, and the presence of inhibitory substances. Regarding external factors, proper hygiene practices and production standards play a critical role, particularly focusing on the initial microbial contamination of raw meat and maintaining appropriate temperature conditions throughout the hygiene chain (Kordiovská *et al.*, 2006).

Biogenic amines (BAs) in seafood have been the focus of significant research due to their unpleasant odour and harmful health effects. While low concentrations of BAs can be decomposed by enzymes such as monoamine oxidase and diamine oxidase in the human intestine, excessive intake of BAs can lead to various health issues, including headaches, diarrhoea, high blood pressure, and heart palpitations (Henríquez-Aedo *et al.*, 2018). Histamine, tyramine, cadaverine, and putrescine are the most commonly studied biogenic amines. Histamine and tyramine are being

linked to "scombroid fish poisoning" and the "cheese reaction," respectively (Gardini *et al.*, 2016). Moreover, while putrescine and cadaverine are less toxic, they can worsen the effects of histamine and tyramine by inhibiting the enzymes responsible for detoxification. Consumption of biogenic amines at low concentrations does not pose a significant health risk to healthy individuals, but at higher concentrations, foodborne intoxication can occur (Bjornsdottir *et al.*, 2009; Noori *et al.*, 2018). The determination of biogenic amines in food products is of ongoing interest due to their undesirable effects on humans, such as rashes, migraines, hypertension, and hypotension (Jiang *et al.*, 2014). Additionally, polyamines are considered potential precursors of carcinogenic nitrosamines (Karovičová, Kohajdová, 2005).

The common carp (*Cyprinus carpio*) is a freshwater species abundant in Western Europe and Central Asia, and it ranks among the most commercially significant species for freshwater aquaculture (Winker *et al.*, 2010). It is one of the most consumed fish species in Central Europe. It is popular for its tender and easily digestible meat with high nutritional value. It is sold fresh and frozen, either whole, filleted, or sliced. Carp meat can be further processed into food products, which broadens the range of uses for this raw material (Křížek *et al.*, 2015). However, as a raw material, it is prone to rapid spoilage (Noori *et al.*, 2018). The concentrations of biogenic amines in fresh fish are very low, but they increase during storage (Veciana-Nogués *et al.*, 1997). Histamine and tyramine pose the highest toxicological risk (Kordiovská *et al.*, 2006).

Quality of meat, dairy products and fish can be evaluated based on biogenic amines (Costa *et al.*, 2018). Current EU legislation addressing microbial criteria for biogenic amines in foods is limited to histamine in fishery products. There are no specific regulations covering other biogenic amines or non-fishery food items. In the EU, the European Commission Regulation (EC) No 2073/2005, along with its amendments such as Regulation (EC) No 1441/2007 and Regulation (EU) No 365/2010, sets safety standards for histamine in two categories of fishery products. For certain fish species (from families like *Scombridae*, *Clupeidae*, and others), histamine levels in nine sampled units are allowed to be between 100 and 200 mg/kg in two units, but none should exceed 200 mg/kg. The same applies to fermented fish products in brine, with permissible histamine limits between 200 and 400 mg/kg in two units, but none exceeding 400 mg/kg. Both cases require

analysis using HPLC, as specified in the regulation (EFSA, 2011). In 2013, Commission Regulation (EU) No 1019/2013 of 23 October 2013 amended Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products, which amended permissible histamine limit in fish sauce produced by fermentation of fishery products to 400 mg/kg in products placed on the market during their shelf-life<sup>7</sup>.

However, histamine is commonly used as a quality indicator for fish that are rich in histidine, such as dark-muscle fish, several approaches were published regarding to evaluation of quality of fish and seafood. In contrast, putrescine and cadaverine serve as more reliable indicators of quality in histidine-poor fish, like white-muscle fish, as well as in shellfish and fermented seafood products (Prester, 2011). Wang et al. (2019) proposed quality indicator as a sum of putrescine and cadaverine in *Pseudosciaena crocea*. Křížek et al. (2018) observed putrescine, cadaverine and tyramine to increase in common carp meat in relation with development of decomposition of tissues, and they recommended not to use polyamines into meat quality consideration. Deng et al. (2024) confirmed that 4 biogenic amines (cadaverine, histamine, tyramine, and putrescine) play role as the dominant biogenic amines in fish contributing to the 92.5–99.9 % of total biogenic amines. The aim of this work was to determine and compare the concentrations of selected biogenic amines, and amino acids, the variability of biogenic amines in relation to their precursors in common carp meat after heat treatment and frozen storage from the point of view to consumer safety.

**MATERIAL AND METHODS**

**Experimental settings**

The experiment used samples of common carp (*Cyprinus carpio*), farmed in Slovakia, provided by METRO Cash & Carry SR s.r.o. Two individuals weighing 2000 g, of the same age and origin, eviscerated, and chilled were used for the analysis. Fillets without skin were analysed in different variants of heat treatment and storage. The experimental variants were as follows: fresh – fresh fillets processed on the day of delivery (13.11.2023), with samples subsequently lyophilised (temperature -50°C, pressure max. 0.05 mBar); baked – heat treatment of fresh muscle by baking at 180°C for 15 minutes. Samples were sealed in roasting bags throughout the baking process. After cooling, the samples were lyophilised; frozen for 1 month – storage of fresh muscle by freezing in vacuum-sealed bags at -18°C for 1 month, followed by lyophilisation; frozen for 3 months – storage of fresh muscle by freezing in vacuum-sealed bags at -18°C for 3 months, followed by lyophilisation. The freeze-dried material was used for preparation of 2 parallel samples from each variant for the measurements of amino acids and for the biogenic amines, as well.

**Analytical procedures and statistical analyses**

The determination of biogenic amines followed the procedure and conditions published in the work of Jakobová et al. (2023), with slight modifications, using homogenised freeze-dried material in the amount of 2.0000±0.0001 g. In brief: a supernatant was prepared from the samples, which was alkalinised and derivatized using dansyl chloride, followed by incubation, liquid-liquid extraction using heptane, evaporation, and reconstitution in the mobile phase – acetonitrile. From each variant, 2 parallel samples for biogenic amines analysis were produced and measurement was performed in 3 replicates for each vial. Analysis was performed using the HPLC-DAD method on an Agilent 1260 Infinity II instrument (Agilent Technologies, Germany). Separation was carried out on a Zorbax Eclipse XDB C18 150 mm x 3.0 mm x 3.5 µm column (Agilent Technologies, USA) with an Agilent EC-C18 30 mm x 4.6 mm x 2.7 µm guard column (Agilent Technologies, USA) at a flow rate of 0.6 ml/min of the mobile phase – water and acetonitrile under gradient elution. Data collection was done at 254 nm. Data summarisation and table preparation were performed in Excel (Microsoft, Redmond, WA, USA), and the Tukey pairwise test was used for statistical analysis in the freely available software Past 4.03 (Hammer et al., 2001). The level of statistical significance was set at p < 0.05 for all analysed biogenic amines.

For amino acid analysis, 0.5 g of pulverized, freeze dried samples from each experimental variant were accurately weighed. The samples were subjected to acid hydrolysis (with HCl c=6 mol/l) for 24 hours at 110°C. From each variant, 2 parallel samples of hydrolysates were produced and measurement was performed in 3 replicates for each vial. Amino acid analyses was conducted using an AAA 400 automatic amino acid analyser (INGOS a.s., Prague, Czech Republic). Sodium-citrate buffer was used as a mobile phase and chromatographic column with catex (OSTION LG ANB, Czech Republic) were used for the separation of amino acids. Derivatization was performed with reaction with ninhydrin, the separated amino acids were detected and data processing was performed by AMIK software 3.0 (Czech Republic) and standard amino acid mixtures used as external standards. The content of individual amino acids was then calculated in grams per 1 kg dry weight (g/kg).

Concentration of individual biogenic amines and a total content of biogenic amines (as a sum of detected biogenic amines putrescine, tyramine, spermidine, and spermine) were calculated in mg/kg DW. Limits of the method (limit of detection and limit of quantification) were tested in our previous work of Jakobová et al.

(2024). Concentration of 14 essential and nonessential amino acids were expressed in g/kg DW. Evaluation of obtained data was performed in Excel software to provide a descriptive statistics and for visualisation of percentage distribution of BAs in individual experimental variants. The software Past 4.03 (Hammer et al., 2001) was used for correlation of amino acids and biogenic amines followed by graphical visualisation in Excel. Parameters were tested for a data normality followed by ANOVA and Tukey HSD tests, based on the results of Shapiro-Wilk normality test. Furthermore, discriminant analysis (DA) was employed to the group of variables, represented by individual amino acids and biogenic amines.

**RESULTS AND DISCUSSION**

Amino acid profile was determined for individual experimental variants. The results of the content of fifteen amino acids are listed in the Table 1, and the presence and abundance of individual amino acids was in agreement with investigation of Buchtová et al. (2009). The effect of heat treatment was visible in differences in the levels of 14 amino acids, belonging to the group of essential and non-essential amino acids. Trend showed similarities for the fresh and frozen samples, stored for different periods and differences especially between fresh and baked samples. This trend was confirmed by ANOVA test on a level of significance p<0.05 for amino acids: aspartic acid, threonine, serine, glutamic acid, glycine, leucine, tyrosine, lysine and arginine. The most abundant amino acid was glutamic acid, which ranged from 53.3±0.9 to 60.2±0.5 g/kg DW. The lowest concentration of glutamic acid was observed in the variant with baked meat.

**Table 1** Concentration of individual amino acids (g/kg DW) in carp meat

| Amino acid    | Fresh                    | Frozen storage 1 month   | Frozen storage 3 months  | Baked                    |
|---------------|--------------------------|--------------------------|--------------------------|--------------------------|
|               | mean±SD                  | mean±SD                  | mean±SD                  | mean±SD                  |
| Threonine     | 17.1±0.7 <sup>a</sup>    | 17.0±0.5 <sup>a</sup>    | 17.2±0.4 <sup>a</sup>    | 14.9±0.5 <sup>b</sup>    |
| Valine        | 26.7±0.6 <sup>a</sup>    | 24.7±0.7 <sup>b</sup>    | 24.7±0.5 <sup>b</sup>    | 21.5±0.9 <sup>c</sup>    |
| Isoleucine    | 16.4±1.2 <sup>a</sup>    | 16.5±1.3 <sup>a</sup>    | 16.6±0.8 <sup>a</sup>    | 14.9±0.8 <sup>a</sup>    |
| Leucine       | 30.4±0.6 <sup>a</sup>    | 31.1±0.7 <sup>a</sup>    | 31.1±0.9 <sup>a</sup>    | 27.2±1.3 <sup>b</sup>    |
| Phenylalanine | 17.9±0.9 <sup>a</sup>    | 17.9±1.1 <sup>a</sup>    | 17.9±0.8 <sup>a</sup>    | 15.6±0.9 <sup>a</sup>    |
| Lysine        | 36.9±1.2 <sup>a</sup>    | 36.9±0.8 <sup>a</sup>    | 37.2±1.3 <sup>a</sup>    | 32.7±1.0 <sup>b</sup>    |
| Histidine     | 14.4±0.8 <sup>a</sup>    | 14.5±0.6 <sup>ab</sup>   | 14.6±0.9 <sup>ab</sup>   | 12.5±0.6 <sup>b</sup>    |
| Arginine      | 21.0±1.0 <sup>a</sup>    | 20.9±0.8 <sup>a</sup>    | 20.3±0.9 <sup>a</sup>    | 14.4±0.7 <sup>b</sup>    |
| Methionine    | 18.8±0.7 <sup>a</sup>    | 18.8±0.9 <sup>a</sup>    | 19.1±1.2 <sup>a</sup>    | 17.3±1.1 <sup>a</sup>    |
| Sum of EAA    | 197.58±6.29 <sup>a</sup> | 200.19±6.04 <sup>b</sup> | 198.74±6.29 <sup>a</sup> | 171.02±6.37 <sup>c</sup> |
| Aspartic acid | 37.2±0.6 <sup>a</sup>    | 36.1±0.1 <sup>a</sup>    | 36.2±0.4 <sup>a</sup>    | 31.3±0.5 <sup>b</sup>    |
| Serine        | 16.6±0.7 <sup>a</sup>    | 16.8±0.6 <sup>a</sup>    | 16.9±0.7 <sup>a</sup>    | 14.5±0.6 <sup>b</sup>    |
| Glutamic acid | 60.2±0.5 <sup>a</sup>    | 60.1±0.8 <sup>a</sup>    | 59.8±0.6 <sup>a</sup>    | 53.3±0.9 <sup>b</sup>    |
| Glycine       | 19.2±0.3 <sup>a</sup>    | 19.5±0.7 <sup>a</sup>    | 20.0±1.1 <sup>a</sup>    | 14.6±0.8 <sup>b</sup>    |
| Alanine       | 21.2±1.0 <sup>a</sup>    | 21.2±0.9 <sup>ab</sup>   | 21.3±0.9 <sup>ab</sup>   | 18.7±1.1 <sup>b</sup>    |
| Tyrosine      | 12.6±0.5 <sup>a</sup>    | 12.6±0.6 <sup>a</sup>    | 12.5±0.7 <sup>a</sup>    | 10.7±0.6 <sup>b</sup>    |
| Sum of NEAA   | 166.99±2.94 <sup>a</sup> | 166.32±3.02 <sup>a</sup> | 166.70±3.59 <sup>a</sup> | 143.12±3.67 <sup>b</sup> |

**Legend:** Content of individual amino acids in dry weigh in g/kg; <sup>a, b, c</sup>—superscript letters indicate significant differences between control variant (fresh) and other experimental variants with different storage times and heat treated meat within one row, p<0.05 (one-way ANOVA, Tukey HSD test), EAA – essential amino acids, NEAA – non-essential amino acids, DW – dry weight, SD – standard deviation.

Changes during the freeze storage was observed to be significant in case of alanine, valine and histidine. In these amino acids was observed slight decrease in concentration. Baking was found to have impact on decrease of all determined amino acids. In case of methionine, isoleucine and phenylalanine, no significant changes were observed between individual variants.

Some types of meat including poultry and fish undergo rapid decomposition, during which free amino acids are released as a result of proteolysis, serving as precursors for the formation of biogenic amines (Wójcik et al., 2023; Dalle Zotte et al., 2020). Key biogenic amines found in seafood include histamine, tyramine, tryptamine, putrescine, and cadaverine, which are derived from the free amino acids histidine, tyrosine, tryptophan, ornithine, and lysine, respectively. Additionally, spermidine and spermine are formed from putrescine. The concentration of free amino acids, particularly histidine, is crucial as it serves as the precursor for histamine biosynthesis (Biji et al., 2016). Individual concentrations of histidine in the carp meat ranged from 12.5±0.6 to 14.6±0.9 g/kg. Histidine content increased slightly with the length of frozen storage, but with the high temperature content of histidine decreased; however, concentration of histamine still did not exceed detection limit (LOD =0.020 µg/mL for liquid extracts) of the HPLC method in this study. The results of frozen storage and baking on the concentrations of biogenic amines in the meat of common carp were evaluated. Among the eight biogenic amines analysed, we found the presence of

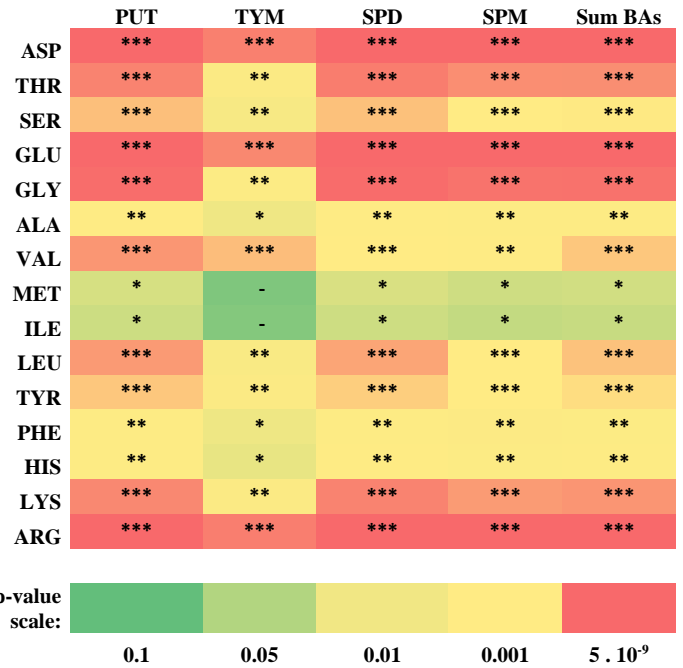
putrescine and spermidine in all experimental variants, with concentrations increasing with the length of freezing storage and also during heat treatment by baking. In fresh common carp meat samples, only putrescine and spermidine were detected and quantified. In samples stored frozen for one month, spermine was identified, and after three months of freezing, tyramine was present. The samples from the individual experimental variants showed in mentioned biogenic amines significant differences marked with superscript letters in Table 2, however, present tyramine shown no significant difference between variants fresh meat and frozen storage for 1 month. Baked fish meat shown significant increase in all biogenic amines, supported by statistical differences. In the other variants, statistically significant differences were found in the levels of putrescine and tyramine.

**Table 2** Concentration of individual biogenic amines (g/kg DW) in carp meat

| Biogenic amines | Fresh                 | Frozen storage 1 month | Frozen storage 3 months | Baked                  |
|-----------------|-----------------------|------------------------|-------------------------|------------------------|
|                 | mean±SD               | mean±SD                | mean±SD                 | mean±SD                |
| Putrescine      | 2.8±0.1 <sup>a</sup>  | 3.1±0.0 <sup>ab</sup>  | 4.3±0.1 <sup>ab</sup>   | 12.3±0.2 <sup>c</sup>  |
| Tyramine        | ND <sup>a</sup>       | ND <sup>a</sup>        | 15.0±0.3 <sup>b</sup>   | 31.4±0.3 <sup>c</sup>  |
| Spermidine      | 15.2±0.1 <sup>a</sup> | 28.8±0.4 <sup>b</sup>  | 32.3±0.2 <sup>b</sup>   | 136.2±1.1 <sup>c</sup> |
| Spermine        | ND <sup>a</sup>       | 16.6±0.4 <sup>b</sup>  | 20.8±0.6 <sup>b</sup>   | 104.9±0.2 <sup>c</sup> |

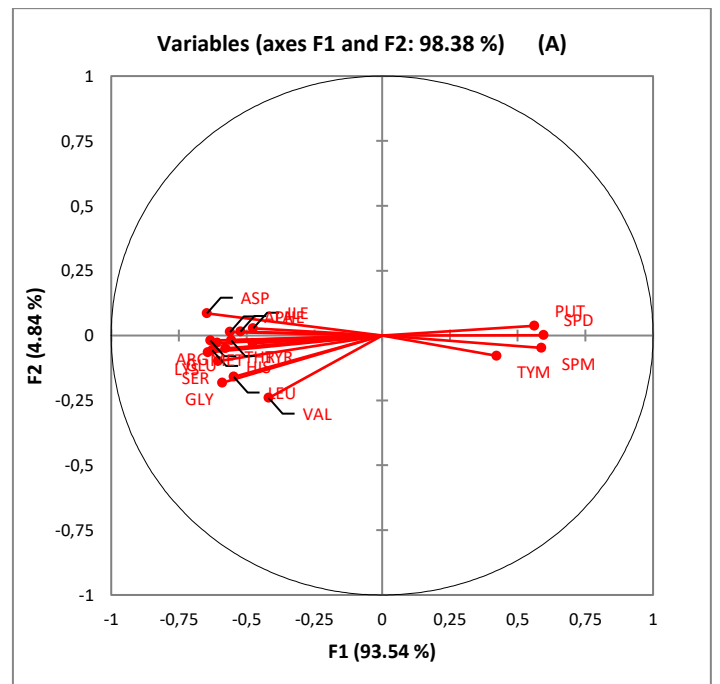
**Legend:** Content of individual biogenic amines in dry weigh in mg/kg; <sup>a, b, c</sup>—superscript letters indicate significant differences between control variant (fresh) and other experimental variants with different storage times and heat treated meat within one row, p<0.05 (one-way ANOVA, Tukey HSD test), DW – dry weight, SD – standard deviation.

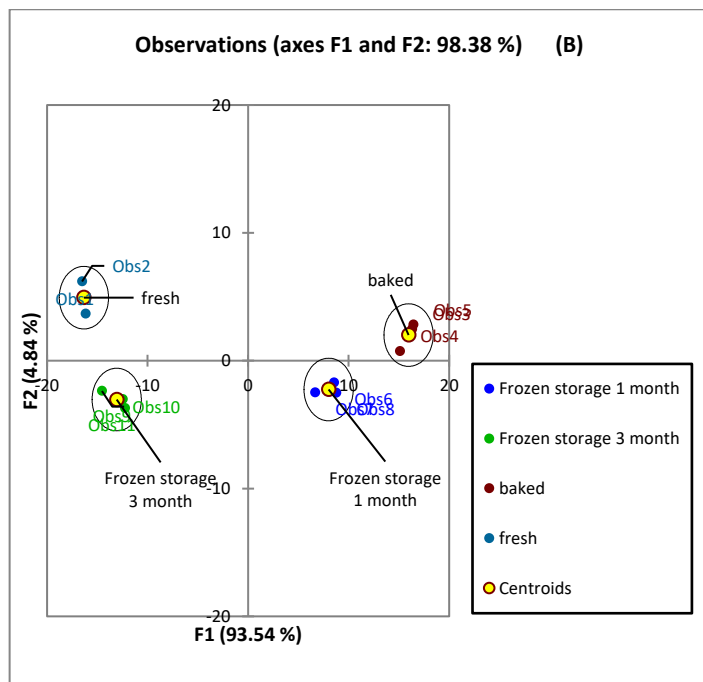
Wang et al. (2019) explored formation of biogenic amines regarding to their dynamic concentrations in relation with free amino acids and proposed sum of putrescine and cadaverine as novel indicator of quality. In the meat subjected to baking, we observed the highest concentrations of biogenic amines compared to the other experimental variants. It can be stated that heat treatment led to an increase in the concentration of selected biogenic amines, with the total amount of biogenic amines rising from the original fresh, uncooked muscle variant at 18.02±0.11 mg/kg to 179.9±1.52 mg/kg, representing nearly a tenfold increase. The impact of different storage temperatures (3±2°C, 24±1°C, and -18±1°C) on the formation of biogenic amines in the muscle tissue of four hybrid lines of carp (*Cyprinus carpio*), in relation to microbial contamination, was studied by Kordiovská et al. (2006). Similar to our study, the authors reported detectable levels of putrescine, spermidine, and spermine, while histamine and tyramine were not detected in carp meat even after 3 months of frozen storage. The authors recommend the combined levels of putrescine and cadaverine as the most objective indicator of meat quality, as their content best correlates with bacterial growth. In our research, cadaverine was not detected in the samples, thus above mentioned indicator was not possible to express. Later formation of cadaverine was documented in the study of Zhang et al. (2015). In their study, during the storage of carp fillets at 20°C and 0°C for 48 hours, six biogenic amines were identified at various times (2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine and spermine; however, cadaverine was in detectable levels after 24 hours at 20°C and after 36 hours at 0°C, was only detected later in the storage duration. Amines in food are typically present towards the end of their shelf life, making the concentrations of biogenic amines a useful indicator of fish quality (Özogul et al., 2006). The effect of frying and baking on the changes in the biogenic amines was investigated on *Dosidicus gigas* by Ning et al. (2022) resulting in the increasing trend of histamine and cadaverine content during the increasing length of these thermal treatments. Similarly, we observed the high increase of biogenic amines in the carp muscle in the baked samples. The total content of biogenic amines in mackerel showed also a significant increase as the processing temperature rose during roasting, frying, and stewing, aligning with findings by Kim et al. (Kim et al., 2021). Makhmureang et al. observed that blanching and boiling elevated the levels of biogenic amines in undried *Hericium erinaceus* samples, in addition to aquatic products (Makhmureang et al., 2021). The explanation of the increase of biogenic amines formation with the application of high temperature culinary treatments can be explained by greater rise of precursor transformation at high heat treatment (Oracz, Nebesny, 2014; Kim et al., 2021). Fatty food during the heat treatment releases lipid peroxides that influences chemical decarboxylation of amino acids (Zamora et al., 2012). Histamine was found under the detection limit in our study, and was not included to evaluation of biogenic amines. The low occurrence of histamine may be linked to the deamination of histidine preferred to its decarboxylation (Ruiz-Capillas, & Moral, 2002). However, histamine is considered as the most toxicologically important indicator of food quality and freshness, common carp meat, stored also in the study of Zhang et al. (2015) showed increasing levels of histamine after 36 hours even at 20°C of storage. The levels of histamine in fillets stored at 20°C and 0°C were significantly lower than limits for histamine content for fish and fishery products (200 mg/kg) (Commission Regulation (EU) No 1019/2013 and Commission Regulation (EC) No 2073/2005).



**Figure 1** Correlation between individual amino acids and biogenic amines  
**Legend:** \* p<0.05; \*\* p<0.01; \*\*\* p<0.001; - p>0.05; ASP—aspartic acid; THR—threonine; SER—serine; GLU—glutamine; GLY—glycine; ALA—alanine; VAL—valine; MET—methionine; ILE— isoleucine; LEU— leucine; TYR— tyrosine; PHE— phenylalanine; HIS— histidine; LYS— lysine; ARG —arginine; PUT—putrescine; TYM—tyramine; SPD—spermidine; SPM—spermine; BAs—biogenic amines.

Only negative correlations in carp tissues were observed for biogenic amines–amino acids as visualized in the figure 1. In all but two cases (methionine vs. tyramine and isoleucine vs. tyramine), were found the correlations on three levels of significance. It is important to mentioned that these four biogenic amines have precursors as follows: Putrescine is produced from ornithine which is synthesized from arginine (Biji et al., 2016). In our study, ornithine was not determined, but arginine as an essential amino acid is in negative correlation with putrescine with significant value p=2.0 10<sup>-7</sup>. Spermidine and spermine are produced from putrescine, so arginine is also a first precursor in this biosynthesis. Spermidine and spermine have in this case negative correlation with arginine with significant p values 3.0 10<sup>-7</sup> in case of arginine vs. spermidine, and 8.4 10<sup>-7</sup> for arginine vs. spermine. Tyrosine is a precursor for tyramine. Correlation analysis showed strong negative correlation, characterized with significant p=0.0033.





**Figure 2** Discriminant analysis map of experimental variants (A-variables; B-observations)

**Legend:** blue—fresh; dark blue—frozen storage 1 month; green—frozen storage 3 month; red—baked variant; ASP—aspartic acid; THR—threonine; SER—serine; GLU—glutamine; GLY—glycine; ALA—alanine; VAL—valine; MET—methionine; ILE—isoleucine; LEU—leucine; TYR—tyrosine; PHE—phenylalanine; HIS—histidine; LYS—lysine; ARG—arginine; PUT—putrescine; TYM—tyramine; SPD—spermidine; SPM—spermine.

Discriminant analysis showed total observation on F1 and F2 axes to be 98.38 %. Eigenvectors were positive for F1 for glutamine, aspartic acid, glycine, valine and spermidine. Negative values were calculated for leucine and isoleucine, other variables had value. For F2 positive eigenvectors were found for aspartic acid, glutamine and spermidine, and negative for glycine, valine, leucine and isoleucine, other variables had the value 0. The cross-validation prior and posterior classification showed the membership probabilities of groups Frozen storage 3 months with baked samples and fresh and frozen storage 1 month, however the centroids of DA biplot showed good discrimination of individual variants to the separate groups.

The Commission Regulation (EU) No 1019/2013 (23 October 2013) and Commission Regulation (EC) No 2073/2005 (15 November 2005), regulate histamine content and set limits specifically for fish and fishery products (200 mg/kg) and fermented fish products (400 mg/kg). However, no official limits are established for biogenic amines or histamine in other food products, although some safety guidelines suggest safe consumption levels per meal for a healthy individual: 50 mg of histamine and 600 mg of tyramine (EFSA, 2011). In our study, no variant of common carp meat, fresh, frozen storage and baked, contained histamine nor cadaverine in dry weight basis. Only putrescine was present in the samples, but its content was maximum 12.4 mg/kg DW. Considering proposed indicators for evaluation of fish quality, sum of putrescine and cadaverine was not applicable due to absence of cadaverine. In our study it was observed that high temperatures have impact on increased levels of putrescine as well as other biogenic amines. Baking increased total content of biogenic amines almost 10-times compared to storage in freezer for 1 and 3 months. Frozen storage increased total content of biogenic amines 1.8 and 2.9-times after 1 and 3 months, respectively, compared to fresh meat in DW basis. Monitoring of biogenic amines levels in foods is important for evaluating potential health risks, especially for sensitive individuals, however in our study, common carp has been identified as safe for consumption under above mentioned frozen storage and heat treatment. Given that foods can contain high levels of histamine and tyramine without obvious changes in taste, smell, or appearance, ensuring food safety by monitoring biogenic amine levels remains a challenging but essential task.

## CONCLUSION

Biogenic amines were present in the fresh meat, and their levels increased during storage, depending on the duration of storage, and also rose with cooking. Notably, concentrations of putrescine, spermidine, spermine, and tyramine were detected. At lower storage temperatures, in accordance with legislative requirements, the levels of biogenic amines complied with the standards set by current regulations. The concentrations of individual biogenic amines in meat stored frozen for one month did not differ from those found in fresh muscle tissue. The total amount of biogenic amines increased almost tenfold with baking probably due to the formation of BA precursors and decarboxylation of aminoacids. The overall

concentrations of biogenic amines did not exceed 200 mg/kg. Histamine was not detected in any of the experimental variants.

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