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Dynamics of the biogenic amines formation in sausages with 45 % of the common carp (*Cyprinus carpio* L.) meat

Dynamik der Bildung biogener Amine in Würsten mit 45 % Fleischanteil vom Karpfen (Cyprinus carpio L.)

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Summary

The aim was to compare the biogenic amines (BAs) content in sausages with different processing (Control/pork sausages, Fish/experimental sausages with 45 % carp meat/) and packaging characteristics (Air/Vacuum) stored for 30 days at 2 ± 2 °C. Samples were taken on the 1st day of production and during the experiment (Days 6, 9, 13, 16, 20, 23, 27 and 30). Measurement was based on high-performance liquid chromatography coupled with tandem mass spectrometry. The total BAs content (mg/kg) in unpacked (Fish/Air: 86.20 ± 2.83) and packed (Fish/Vacuum: 92.63 ± 4.53) sausages was higher than that in the pork sausages (Control/Air: 74.16 ± 2.04, Control/Vacuum: 75.60 ± 2.46). The greatest proportion of BAs (mg/kg) was made up of spermine (Fish/Air: 79.48 ± 2.59, Fish/Vacuum: 87.26 ± 4.78, Control/Air: 69.49 ± 2.00, Control/Vacuum: 70.73 ± 3.73). Histamine was present at a quantity of less than 0.5 mg/kg. Cadaverine, tryptamine and 2-phenylethylamine were not detected in any of the groups of samples.

Keywords: Fish, Meat product, Histamine

Zusammenfassung

Ziel der Arbeit war, die Dynamik der Bildung von biogenen Aminen (BA) in unverpackten und vakuumverpackten Bratwürsten mit 45% Fleischanteil vom Karpfen (*Cyprinus carpio* L.) zu erfassen, die in warmem Rauch geräuchert und 30 Tage lang bei einer Temperatur von +2 ± 2 °C gelagert wurden (Experimentproben: Fish/Air resp.Vacuum). Als Kontrollproben dienten unverpackte und vakuumverpackte Bratwürste aus Schweinefleisch (Control/Air resp. Vacuum). Analysiert wurden die Proben unmittelbar nach der Herstellung (am 1. Tag) und dann im Laufe des Lagerungsexperiments (am 6., 9., 13., 16., 20., 23., 27. und 30. Tag). Zur Bestimmung der biogenen Amine wurde Hochleistungsflüssigkeitschromatographie in Verbindung mit Tandem-Massenspektrometrie eingesetzt. Der Gesamtgehalt biogener Amine (mg/kg) in den Bratwürsten mit 45 % Karpfenfleischanteil (Fish Air: 86.20 ± 2.83/Vacuum: 92.63 ± 4.53) war im Vergleich zu den Kontrollwürsten (Control Air: 74.16 ± 2.04/Vacuum: 75.60 ± 2.46) höher, unabhängig von der Art der Verpackung/Lagerung. Am meisten (mg/kg) war Spermin enthalten (Fish/Air: 79.48 ± 2.59, Fish/Vacuum: 87.26 ± 4.78, Control/Air: 69.49 ± 2.00, Control/Vacuum: 70.73 ± 3.73), Histamin lag unter 0.5 mg/kg. Kadaverin (0.192), Tryptamin (0.0092) und 2-Phenylethylamin (0.0055) waren in Mengen enthalten, die unter dem Detektionslimit lagen (Wert in mg/kg in Klammern). Die Arbeit wies nach, dass die Aufnahme von biogenen Aminen, insbesondere Histamin, durch den Verzehr von Bratwürsten mit 45 % Fleischanteil vom Karpfen vergleichbar ist mit jenem beim Verzehr traditioneller Bratwürste aus Schweinefleisch. Der Verzehr von Bratwürsten mit Karpfenfleischanteil ist als sicher für die Gesundheit des Menschen anzusehen.

Schlüsselwörter: Fisch, Fleischprodukt, Histamin

Introduction

Biogenic amines (BAs) are non-volatile low-molecular-weight nitrogenous organic bases formed by the decarboxylation of corresponding amino acids (Křížek et al., 2002; Křížek et al., 2004). The enzymes responsible, amino-acid decarboxylases, are raw material and in microorganisms (Karovičová and Kohajdová, 2005).

Scombroid fish of the families *Scombridae, Clupeidae* and *Engraulidae* associated with a large amount of free histidine have been reported to contain considerable levels of BAs, especially histamine (Jairath et al., 2015). Many papers report results on the BAs in different types of products, demonstrating a great variability in their amounts (Jairath et al., 2015). Large variations can be observed in the content in different batches of the same commercial product (Ansorena et al., 2002). The level of BAs is greatly influenced by pH, as the pH decreases, decarboxylase activity increases and thereby increases the production of BAs (Jairath et al., 2015). BAs are thermo-stable and are not inactivated by heat treatment (Hernández-Jover et al., 1996). The effect of freezing and storage at around –20 °C on the formion of BAs in fish meat has been published by Emborg et al. (2002). The certain delay of histamine formation in thawed fish, when compared with fresh fish, has been explained by the inactivation of psychrotolerant and strongly histamine-producing bacteria during freezing (Dalgaard et al., 2006). The effect of vacuum-packaging on BAs formation has been studied using chilled raw pork tenderloin samples (Li et al., 2014). Biogenic amines in raw common carp *(Cyprinus Carpio)* meat do not represent any hazard to consumers (Křížek et al., 2002). The effect of non-vacuum/vacuum packaging on BA formation in the chilled flesh, minced flesh and fillets of common carp has been published by Křížek et al. (2004; 2011). Meat is the raw material that contributes to the final BAs content in cooked meat products. The presence of BAs in cooked meat products can be the result of the use of meat of poor hygiene quality (Hernández-Jover et al., 1996). Several studies have been carried out using BAs as indicators of sausage safety and quality during storage (Eerola et al., 1997; Demeyer, 2002; Hu et al., 2007; Xu et al., 2010). High levels of BAs have been detected in traditional Chinese sausages manufactured by spontaneous (uncontrolled) fermentation (Lu et al., 2010).

The aim of this paper was to compare the biogenic amine content (sum of the BAs spermine, putrescine, spermidine, histamine and tyramine) in four types of sausage samples with different processing (pork Control/Fish experimental) and packaging (unpacked Air/Vacuum-packed) characteristics stored for 30 days at 2 ± 2 °C.

Materials and Methods

Production technology

Fillets of common carp (*Cyprinus carpio* L.) were used for the production of experimental sausages with 45 % fish meat (the breeder: Rybníkářství Pohořelice comp., Pohořelice, Czech Republic, the vendor: Fish shop Josef Šopík, Brno, Czech Republic). The experimental sausages were made at the Technology Workshop at the Department of Meat Hygiene and Technology at the Faculty of Veterinary Hygiene and Ecology of the University of Veterinary and Pharmaceutical Sciences in Brno. Preparation of raw materials: 1 kg of 45 % sausages: 45 % fish + 55 % pork (the term fish means carp fillet, while the term pork means 90 % of only lean pork shoulder and 10 % pork back fat). Additives and seasoning mix: nitrite salting mix E250 (180 g/kg), garlic (16 g/kg), antioxidant sodium erythorbate E315 (1 g/kg) and the seasoning mix SCZA 04006 Sausage Franta Excelent Solo (4 g/kg) from the company TRUMF International s.r.o. (Dolní Újezd, CR); potable water 0.1 l/kg was also used. Control samples of sausage (0 %) were made without fish meat (100 % pork). Pig intestine served as a casing. The samples of sausages were made using an ordinary technological procedure that consists of the following steps: preparation and weighing of raw materials, grinding, salting, mixing, filling, cooking at 70 °C for 10 minutes, smoking (beech wood) for 2.5 hours, cooling with cold water (Kašpar and Buchtová, 2015). Samples were collected from sausages on the 1st day of their production and every three to four days of storage during the experiment (Days 6, 9, 13, 16, 20, 23, 27 and 30).

Biogenic amine analysis

Measurement of BAs was based on high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry. Homogenised samples (0.5 g) were weighed in a 10 ml glass tube and extracted with a 5 % trichloroacetic acid solution in water. One-step extraction for 20 minutes followed by a clean-up step using a 0.45 µm syringe filter was employed for sample preparation. Biogenic amines were subsequently separated by reverse-phase liquid chromatography using a C column (2.1 mm × 50 mm, 1.9 µm; Thermo, San Jose, CA, USA) and detected by tandem mass spectrometry using heated electrospray ionisation in the positive ion mode. A Thermo Scientific UHPLC Accela 1250 system was connected to a Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, CA, USA). Standards of putrescine, spermidine, tyramine, histamine and spermine, as well as trichloroacetic acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were of residual analysis purity (Chromservis, s.r.o., Czech Republic).

Statistical analysis

The results were evaluated (mean±s.d.) in the program Microsoft Office Excel 2007. Statistically significant differences were performed using UNISTAT 6.0 (Unistat® Limited, London, England). Differences between all days of sampling for sausages under the same conditions of storage (e. g. Control/Air: sum BAs on Days 1, 6, 9, 13, 16, 20, 23, 27 and 30 of sampling) were subjected to a Kruskal-Wallis ANOVA test (in view of the non-normal distribution of data) and, subsequently, to nonparametric Tukey-type multiple comparison tests. Differences between control and experimental samples for the same parameter and day of sampling (e. g. sum of BAs in unpackaged Control/Fish sausages on Day 1 of sampling) were subjected to a Mann-Whitney test. A value of $P<0.05$ was considered significant, and a value of $P<0.01$ was considered highly significant.

Results

The **biogenic amines content** was higher throughout the entire experiment in sausages with 45 % carp meat than in the control samples, with the highest BAs values being found in samples packed in a vacuum (Fish/Vacuum). No

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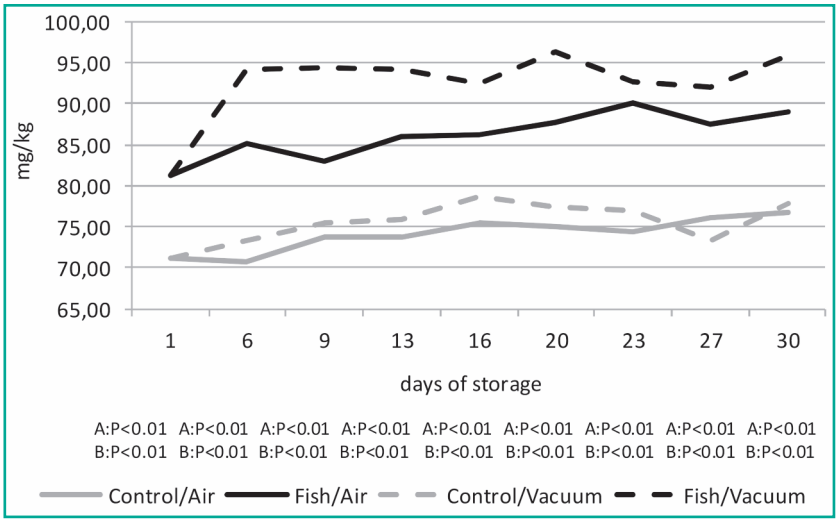

FIGURE 1: *Total content of BAs (in mg/kg) in control pork sausages and in experimental sausages with 45 % carp meat stored for 30 days at 2 ± 2 °C in air and packed in a vacuum. Lines below the x-axis show differences in values (P<0.01) of BAs between Control/Air vs. Fish/Air (group A) and between Control/Vacuum vs. Fish/Vacuum (group B) for the same day of sampling (e.g. Day 1 of storage).*

statistically significant differences (P>0.05) in the BAs content in dependence of the length of the experiment were found in any group of sausages (the differences in values between individual days were practically the same for all groups of sausages). In contrast, highly conclusive differences (P<0.01) in the BAs content were found between the samples Control/Air vs. Fish/Air and the samples Control/Vacuum vs. Fish/Vacuum for every day of sampling (Figure 1).

The largest proportion was comprised of **spermine** (Table 1). The spermine content was higher in sausages with 45 % carp meat throughout the experiment, and its formation was supported by vacuum-packing. No statistically significant differences (P>0.05) in the content of spermine in dependence on the length of the experiment were found in any group of sausages (the differences in values on the individual days were practically the same for each group of sausages). In contrast, highly conclusive differences (P<0.01) in the spermine content were found between the samples Control/Air vs. Fish/Air and the samples Control/Vacuum vs. Fish/Vacuum for every day of sampling.

Putrescine was next BAs that was formed in significant quantities in the samples (Table 1). Its formation was influenced both by the composition of the samples and by the method of packaging of the samples. The largest amount of putrescine was formed in unpacked sausages with 45 % carp meat (Fish/Air), while

TABLE 1: *Content of spermine, putrescine, spermidine, tyramine and histamine and the average content of BAs (in mg/kg) throughout the experiment in pork sausages (Control sample) and in sausages with 45 % carp meat (Experimental sample) stored for 30 days at 2 ± 2 °C in Air and packed in a Vacuum. Table 1 shows differences (P<0.01) in values of spermine, putrescine and spermidine between Control/Air (C/A) vs. Fish/Air (F/A) and between Control/Vacuum (C/V) vs. Fish/Vacuum (F/V) for the same day of sampling (e. g. Day 1 of storage). Statistically significant differences (P<0.01) in values among specific amines in all groups of samples were practically the same (the last column on the right). The numerically lower value is indicated by an "a".*

BAs	Types of samples	Days of storage at 2 ± 2 °C 1	6	9	13	16	20	23	27	30	Mean of BAs	Stat. sign.
Spermine	C/A	66.66±4.69	66.34±3.06	68.46±2.11	69.35±3.35	70.64±2.66	70.55±3.49	69.88±3.02	71.62±1.71	71.91±0.01	69.49±2.00	
	F/A	75.15±2.53	78.05±2.45	76.45±0.93	79.42±1.84	79.52±1.73	80.59±6.38	82.82±7.55	80.68±2.32	82.61±0.01	79.48±2.59	
	Stat. sign.	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	d
	C/V	66.66±4.69	67.42±2.57	69.17±1.66	70.16±2.16	72.67±0.65	79.11±8.06	70.89±0.72	68.38±1.74	72.08±0.01	70.73±3.73	
	F/V	75.15±2.53	88.90±0.57	89.14±1.43	88.72±0.60	86.89±1.10	91.17±1.95	87.63±3.80	87.04±0.45	90.71±0.01	87.26±4.78	
	Stat. sign.	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	
Putrescine	C/A	2.58±0.40	2.51±0.18	3.06±0.25	2.61±0.44	2.73±0.26	2.55±0.34	2.34±0.13	2.56±0.06	2.89±0.30	2.65±0.21	
	F/A	3.88±0.29	4.73±0.23	4.24±0.14	4.58±1.48	4.27±0.48	4.81±0.35	4.96±0.42	4.47±0.74	4.34±0.73	4.48±0.34	
	Stat. sign.	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	c
	C/V	2.58±0.40	3.83±0.86	4.26±0.31	3.73±0.47	3.80±0.71	3.34±0.94	3.86±1.59	2.99±0.60	3.67±1.07	3.56±0.51	
	F/V	3.88±0.29	3.41±0.25	3.44±0.50	3.56±0.48	3.64±0.53	3.42±0.35	3.22±0.20	3.32±0.20	3.37±0.14	3.47±0.20	
	Stat. sign.	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
Spermidine	C/A	1.37±0.04	1.31±0.03	1.54±0.09	1.38±0.02	1.41±0.03	1.36±0.02	1.38±0.19	1.34±0.01	1.32±0.01	1.38±0.07	
	F/A	1.75±0.01	1.91±0.09	1.77±0.08	1.53±0.31	1.90±0.02	1.85±0.09	1.76±0.13	1.90±0.05	1.70±0.01	1.79±0.12	
	Stat. sign.	P<0.01	P<0.01	P<0.01	P>0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	b
	C/V	1.37±0.04	1.49±0.22	1.62±0.22	1.55±0.26	1.62±0.21	1.51±0.35	1.64±0.40	1.45±0.15	1.56±0.01	1.53±0.09	
	F/V	1.75±0.01	1.34±0.21	1.40±0.27	1.35±0.16	1.42±0.24	1.33±0.20	1.33±0.25	1.28±0.18	1.37±0.01	1.40±0.14	
	Stat. sign.	P<0.01	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
Tyramine	C/A	0.25±0.07	0.25±0.04	0.24±0.02	0.23±0.06	0.27±0.03	0.24±0.06	0.23±0.04	0.20±0.03	0.23±0.04	0.24±0.02	
	F/A	0.21±0.02	0.19±0.01	0.20±0.01	0.19±0.04	0.18±0.02	0.21±0.02	0.19±0.01	0.21±0.03	0.19±0.01	0.20±0.01	
	C/V	0.25±0.07	0.18±0.01	0.19±0.01	0.19±0.01	0.18±0.01	0.18±0.01	0.19±0.02	0.19±0.01	0.19±0.01	0.19±0.02	a
	F/V	0.21±0.02	0.16±0.01	0.19±0.02	0.19±0.01	0.14±0.01	0.17±0.01	0.17±0.02	0.15±0.02	0.15±0.01	0.17±0.02	
Histamine	C/A	0.41±0.01	0.37±0.17	0.42±0.08	0.29±0.07	0.36±0.01	0.35±0.10	0.55±0.30	0.45±0.14	0.42±0.45	0.40±0.07	
	F/A	0.22±0.07	0.24±0.09	0.24±0.03	0.28±0.04	0.33±0.06	0.22±0.00	0.29±0.05	0.28±0.03	0.28±0.71	0.26±0.04	
	C/V	0.41±0.01	0.35±0.08	0.32±0.08	0.38±0.06	0.36±0.03	0.36±0.02	0.33±0.02	0.40±0.10	0.38±0.62	0.36±0.03	a
	F/V	0.22±0.07	0.28±0.01	0.34±0.01	0.43±0.05	0.34±0.02	0.31±0.07	0.38±0.14	0.30±0.07	0.36±0.53	0.33±0.06	

No statistically significant differences (P>0.05) were found of tyramine and histamine between C/A vs. F/A; and between C/V vs. F/V for the same day of sampling. P<0.01

the smallest amount was detected in the control samples (Control/Air) that were stored unpacked. No statistically significant differences (P>0.05) in the putrescine content were found in dependence on the length of the experiment. In contrast, highly conclusive differences (P<0.01) in the putrescine content were found between the Control/Air vs. Fish/Air samples. Differences in the values of putrescine between the samples Control/Vacuum vs. Fish/Vacuum from Days 6 to 30 of sampling were not found (P>0.05), with the exception of Day 1 of the study (P<0.01).

The content of **spermidine** was the third largest (Table 1). Similarly as for putrescine, the formation of spermidine was also influenced by the material composition and method of packaging of the samples. The vacuum-packed samples Fish/Vacuum contained significantly less spermidine than the unpacked samples (Fish/Air), while the vacuum-packed pork sausages (Control/Vacuum) contained more spermidine than the unpacked control samples. No statistically significant differences (P>0.05) in the spermidine content in dependence on the length of the experiment were found. In contrast, highly conclusive differences (P<0.01) in the spermidine content were found between the samples Control/Air vs. Fish/Air, with the exception of Day 13 (P>0.05). No differences in the values of spermidine between the samples Control/Vacuum vs. Fish/Vacuum were found from Day 6 to 30 of sampling (P>0.05), with the exception of first Day of the study (P<0.01).

The content of **histamine** was extremely low throughout the experiment in all groups of products (Table 1). The formation of histamine during the experiment was irregular with inconclusive fluctuations of values. The kinetics of histamine formation in the groups Control/Vacuum and Fish/Vacuum was similar. **Tyramine** was the BA formed in the smallest quantities in the samples of sausage (Table 1). Vacuum-packing inhibited its formation in both types of sample (Control/Fish). The kinetics of its formation had a stagnant or gently falling nature. No statistically significant differences (P>0.05) in the histamine or tyramine content were found in dependence on the length of the experiment and, similarly, for the same day of sampling (P>0.05).

Cadaverine (detection limit 0.192 mg/kg), **tryptamine** (0.0092 mg/kg) and **2-phenylethylamine** (0.0055 mg/kg) were not detected in any group of samples.

Discussion

Packaging plays an important role in delaying/accelerating the production of BAs due to inhibition/stimulation of microorganisms or enzymes producing BAs (Jairath et al., 2015). In our experiment, the kinetic of BAs formation was significantly influenced by the addition of fish to the product ingredients and was similar for both kinds (Air/Vacuum) of packaging (Figure 1). The reason for the greater BAs content (P<0.01) in samples of sausages with carp meat, as compared with pork sausages was probably not merely the addition of the fish meat itself, which replaced almost half the weight of the raw material used in the production of the sausages, but primarily the method of its technological processing. The fresh raw skinned fish fillets were, after salting (18 % NaCl solution/12 hours), smoked with hot smoke (75 °C/1 hour/35 minutes) before use as an ingredient to achieve the targeted modification of the technological properties of the meat. The NaCl resulted in changes to the osmotic pressure in intercellular spaces and inside cells and, as a result of this, to the partial loss of freely bound water and the firming of the muscle tissue. Myofibrillar proteins were coagulated by the hot smoke which led to even greater firming of the raw material from the technological viewpoint. From the organoleptic viewpoint, smoking highlighted the specific taste and aroma. The fillets were manually deboned following cooling to remove intermuscular bones, during which individual myomeres of muscle tissue were gently separated from each other with a knife and the visible bones removed from the meat. During this preparation of the fish material, which lasted more than twelve hours, activation of decarboxylation, native and/or bacterial enzymes originating from contaminating microflora probably took place.

Higher BAs values in our sausages, in comparison with the values given for fresh pork (Szerdahelyi et al., 1993) or fish meat (Křížek et al., 2011), may also be associated with their gradual accumulation occurring as a result of changes to the chemical composition. They are also characterised by a change in the proportion of the components moisture/dry matter in comparison with raw meat. In terms of the quantity of BAs, our experiment showed a relatively constant trend with storage time. Spermine was the dominant biogenic amine formed in all groups of sausages (Table 1). During the experiment, its formation was significantly (P<0.01) supported by vacuum packaging, especially in sausages with 45% fish meat. Hernández-Jover et al. (1996) published the finding that only spermine should be present in products if fresh meat is used and no additional formation occurs during manufacture and cooling. Spermidine was present in fairly low amounts and showed a slight decrease with storage time. Low variability of spermidine concentration was found among the four types of sausage samples. The polyamines spermine and spermidine are known to be naturally occurring amines in fresh meat, and their formation is not associated with spoilage. They are not formed by microbial decarboxylation of amino acids, but are formed from putrescine (Hernández-Jover et al., 1996). They are not toxicologically important and their content cannot serve as a quality indicator (Křížek et al., 2004).

The second highest concentration was observed for putrescine, though these values were incomparably smaller than those for spermine (Table 1). Putrescine is associated with the deterioration of microbiological quality in vacuum-packed stored meat (Eerola et al., 1997) and can be produced from three amino acids: glutamine, arginine and agmatine (Stadnik and Dolatowski, 2010). Even though the toxicities of diamines (putrescine and cadaverine) are relatively low, there is evidence to suggest their precursor role in the formation of carcinogenic nitrosamines by reaction with nitrite (Eerola et al., 1997). Putrescine has been reported as a potentiator, with cadaverine, for the toxic effect of histamine and tyramine (Ansorena et al., 2002) which are considered anti-nutritional compounds (Křížek et al., 2004).

Histamine is the amine most studied with regard to its toxicological effects. An intake of 5–10 mg/kg of histamine can be considered dangerous to some sensitive people, 10 mg/kg is considered as the tolerable limit, 100 mg/kg induces medium toxicity and 1000 mg/kg is highly toxic (Karovičová and Kohajdová, 2005). In this study, values determined for histamine and tyramine were very low (Table 1) and no statistically important trend was observed

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during the experiment. Histamine is quantitatively the most important BA formed during fermentation in silver carp sausages (Xu et al., 2010). The dynamics of BA creation in dry sausages of the European type (Eerola et al., 1997) and fermented silver carp sausages inoculated with mixed starter cultures (Hu et al., 2007) have been published. In European ripened sausages, BAs may be formed by the action of microbial decarboxylases on free amino acids, resulting from the proteolytic process that normally takes place during ripening (Ansorena et al., 2002). An increase in the amine content was observed when proteolysis was accelerated using a proteinase (Bruna et al., 2000). Methods for preventing the formation of BAs focus on eliminating and/or deactivating decarboxylating microbes, the use of top-quality raw meat materials, starter cultures with negative-decarboxylate activity, and processing applying good manufacturing practise throughout the process (Hu et al., 2007).

The experiment showed that sausages with 45 % common carp meat may be considered safe food from the viewpoint of intake of biogenic amines, and histamine in particular. During the storage experiment the total BAs content was lower than 100 mg/kg. The largest proportion of BAs was comprised of spermine. The histamine content was negligible from the viewpoint of its significance to human health.

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Conflict of interest

The authors declare that no conflict of interest exists.

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