THE ESTIMATION OF BIOGENIC AMINES IN MEAT BY HPLC METHOD

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SUMMARY

Levels of biogenic amines: tryptamine (TRY), phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), and spermidine (SPD) were estimated by means of the high performance liquid chromatography (HPLC) method in meat. Specimens were taken from the meat used for production of fermented meat products. Additional specimens were taken from fresh chicken breast meat stored at an ambient temperature of 4°C and analysed on day 2 of storage. Obtained results varied from 0 to 20.0 mg x kg^{-1} and they were consistent with literature data. The levels of biogenic amines in poultry meat were lower. Described method is rapid, precise and convenient for biogenic estimation in meat.

Key words: *biogenic amines, meat, HPLC method*

INTRODUCTION

Biogenic amines, from the chemical point of view, are organic low-molecular, alkaline, organic substances of various structures. Amines can by created by amination of aldehydes and ketones or by decarboxylation of the corresponding amino acids. Biogenic amines are natural constituents of metabolism in humans, animals, plants and microorganisms. Under normal conditions they play important roles in organisms; biogenic amines are of great importance for normal functioning of the nervous system, they influence intestine motility, regulate body temperature and circulatory system.

Increase in biogenic amine levels caused by the consumption of food rich in these substances results in increased or decreased blood pressure, temperature, respiratory disturbances, a burning sensation in the mouth, nausea, and migraine.

From the hygienic viewpoint, biogenic amines belong to natural anti-nutritional factors in food and are listed in the *Codex Alimentarius* in the group of endogenous pollutant-substances. Their occurrence in food is either physiological as they are a natural part of cell structure; they may also be formed during the production process or/and during the storage of food as a result of metabolic activities of microorganisms. Because of their possibly high and thus health endangering levels, biogenic amines are considered especially in those foods that are submitted to fermentation or ageing processes with the participation of microflora. It concerns e.g. meat products, fish and fish products, cheese, sauerkraut and other pickled vegetables, beer, wine, yeast and soy sauce. The amount of amines in the above mentioned foodstuffs can be used as a criterion for quality evaluation (Askar and Treptow, 1986, Křížek and Kalač, 1998).

The aim of this study was to introduce into practice the methods for the isolation and determination of biogenic amines in meat and meat products. According to the *Codex Alimentarius* of the Slovak Republic (2003), only the maximum tolerable levels of histamine and tyramine have been laid down and must be estimated in foodstuffs. However, adverse health effects of other biogenic amines are well known and often they act in synergy with histamine/tyramine and therefore, it is appropriate to monitor the levels of these other biogenic amines also. High

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biogenic amine concentrations were found especially in the fermented meat products (Dičáková, 2003), and their levels depended on the quality of used raw meat material (Paulsen and Bauer, 1997).

MATERIAL AND METHODS

Material: The amount of tryptamine (TRY), phenylethylamine (PHE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), and spermidine (SPD) was determined in five groups of meat. Meat samples were taken from the meat used for production of fermented meat products (lean pork, fat pork, bacon, and beef). Meat samples were stored at -30 °C for 3 months. Additional samples of fresh chicken breast meat were taken and stored at 4°C and then analysed on day 2 of storage.

Biogenic amine standards: The mixture of the above mentioned 7 amines was used for standard solutions at 6 concentrations, 5 to 250 µg, of each amine/1 ml. Standards were mixed with dansyl chloride and saturated sodium hydrocarbonate solution ($NaHCO_3$), incubated for 10 minutes at 70 °C, evaporated and directly analysed after dissolution in acetonitrile.

Sample preparation: Biogenic amines were extracted with trichloroacetic acid (TCA) from six individual samples from each meat group according to modified, previously used methods (Mietz and Karmas, 1977, Paulsen and Bauer, 1997). Ten grams of fine ground meat were homogenised with 90 ml of 5% TCA. Samples were centrifuged for 15 minutes at 4 °C and at 3500 rpm. After removal of the fat layer, samples were filtered through a 0.45-µm membrane filter (Whatman, 25 mm GD/X). The obtained extracts were stored in the refrigerator at –20°C until analysis.

After derivation by means of dansyl chloride, the sample extracts were analysed the same way as the standards.

Chromatography: Dansylated biogenic amines were determined by the high performance liquid chromatography (HPLC) method using Hewlett-Packard apparatus, HP series 1050 equipped with quaternary pump and auto-sampler. Peaks of individual amines were detected at 254 nm by an UV detector with variable wavelength and an integrator HP 3396 II. Hypersil BDS C–18 (3 µm, 100 × 4 mm I.D.) column was used for analysis. Amines were eluted from the column by isocratic flow of the three-component mobile phase (methanol : acetonitrile : 0.02 N acetic acid = 32:38:30). The optimal division of the standard mixture of seven selected biogenic amines was achieved at flow rate of 0.7 ml x min^{-1} within 25 minutes.

RESULTS AND DISCUSSION

Sample preparation and the ratio of individual agents used for derivation were set by experimental optimisation. The ratio of mobile phase constituents was also gradually modified to reach symmetrical, sharp peaks at optimum distance by the dilution of the standard mixture. Biogenic amines were eluted in this order: tryptamine (4.02 min), phenylethylamine (4.97 min), putrescine (6.18 min), cadaverine (7.07 min), histamine (8.46 min), tyramine (15.92 min) and spermidine (23.32 min).

A comparison of the peak areas obtained by analysis of the standard mixture at 6 different concentrations is shown in Fig. 1. Calibration lines were drawn for each examined amine. Their equations, standard deviations, and correlation coefficients were calculated (TRY – 0.9998; PHE – 0.9995; PUT – 0.9999; CAD – 0.9999; HIS – 0.9999; TYR – 0.9986; SPD – 0.9992).

Detection limit of 0.2 mg was estimated for all amines. For recovery estimation 100 g samples of lean pork were analysed in three parallels spiked with 50 µg and 500 µg respectively, of each of analysed biogenic amines. Calculated recoveries were as follows: TRY - 66%, PHE –73%, PUT - 101%, CAD - 105%, HIS - 89%, TYR - 82 %, SPD - 113%.

In the experiment, the levels of biogenic amines in four different meats used for production of long lasting thermally untreated meat products were estimated. Additional meat samples were taken from fresh chicken breasts. In total 28 samples were analysed. The permissible levels according to *Codex Alimentarius* of the Slovak Republic were not exceeded in any analysed sample (maximal permissible level of histamine is 200 mg x kg^{-1} for fish and fish products with the stated limit – 100 mg x kg^{-1}; maximal permissible level of tyramine is 200

Fig 1. Comparison of the peaks of biogenic amines in the standard mixture solutions of different concentrations (5 to 250 µg per one ml)
Usporedba rezultata biogenih amina u smjesama standardnih otopina različitih koncentracija (5 do 250 µg po 1 ml)

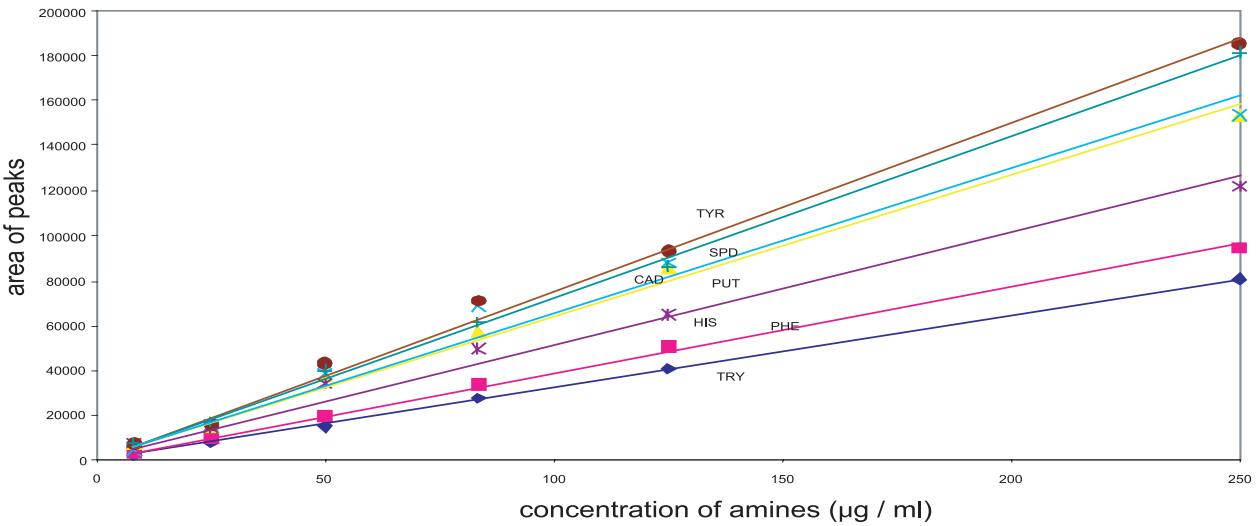


Table 1. Biogenic amine levels in lean pork meat in mg x kg $^{-1}$ (n = 6)
Razine biogenih amina u nemasnoj svinjetini, mg x kg $^{-1}$ (n = 6)

Sample Uzorak	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	0.8	2.1	0.0	0.0	0.0	3.3	2.5
2	1.7	0.9	2.4	0.0	5.3	1.9	2.0
3	0.0	3.2	4.2	0.0	6.0	1.0	0.8
4	0.0	2.5	0.0	3.9	0.4	2.1	3.6
5	0.0	2.7	2.4	2.9	0.0	0.2	2.9
6	0.2	1.1	1.2	2,3	0.0	0.4	3.7
Mean Srednja vrijednost	0,5	2.1	1.7	1.5	2.0	1.5	2.6

Table 2. Biogenic amine levels in fat pork meat in mg x kg $^{-1}$ (n = 6)
Razine biogenih amina u masnoj svinjetini, mg x kg $^{-1}$ (n = 6)

Sample Uzorak	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	0.8	5.1	1.6	2.2	20.0	0.0	1.8
2	0.0	2.0	0.0	0.0	2.4	0.6	2.6
3	0.2	17.2	4.4	0.0	9.5	0.0	0.0
4	1.0	8.3	1.3	1.7	2.9	0.0	3,8
5	1.6	3.5	0.5	0.8	0.4	1.0	1.9
6	0.4	9.7	3.2	2.0	0.0	0.8	2.5
Mean Srednja vrijednost	0.7	7.6	1.8	1.1	5.9	0.4	2.1

▼ **Table 3.** Biogenic amine levels in bacon in mg x kg $^{-1}$ (n = 6)
Razine biogenih amina u slanini, mg x kg $^{-1}$ (n = 6)

Sample Uzorak	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	0.2	0.0	0.0	0.0	0.0	0.0	0.5
2	1.8	0.0	2.4	0.0	0.0	0.0	0.0
3	0.0	0.0	1.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	18,0	2,3	0.0	3,4	0.2
6	0.0	0.0	0.8	0.0	0.0	0.0	0.0
Mean Srednja vrijednost	0.3	0.0	3.7	0.4	0.0	0.6	0.1

▼ **Table 4.** Biogenic amine levels in beef in mg x kg $^{-1}$ (n = 6)
Razine biogenih amina u teletini, mg x kg $^{-1}$ (n = 6)

Sample Uzorak	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	0.0	0.8	0.2	0.0	0.4	2.3	1.4
2	0.0	0.0	0.0	0.0	0.6	1.8	2.0
3	0.0	2.4	0.6	0.8	8.3	3.4	1.8
4	0.0	1.6	0.0	1.7	2.9	1.1	2.5
5	0.0	1.0	0.0	0.9	1.8	0.4	3.1
6	0.6	2.2	1.2	2.1	2.5	1.3	2.4
Mean Srednja vrijednost	0.1	1.3	0.3	0.9	2.8	1.7	2.2

▼ **Table 5.** Biogenic amine levels in poultry meat in mg x kg $^{-1}$ (n = 4)
Razine biogenih amina u oilećem mesu, mg x kg $^{-1}$ (n = 4)

Sample Uzorak	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.5
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.2	0.2
Mean Srednja vrijednost	0.0	0.0	0.0	0.0	0.0	0.1	0.2

mg x kg^{-1} with the stated limit 100 mg x kg^{-1} for hard cheese).

Amine concentrations (as mean values of two determinations) in examined samples are summarised in Tables 1 - 5. Based on the obtained results, it is possible to conclude that high levels of biogenic amines were not found in analysed samples.

In Fig. 2, the levels of individual biogenic amines are shown as estimated in different meat samples.

From the results presented in Tables 1–5 and Figure 2 it is evident that biogenic amines levels in meat are relatively low and on an average they were less than 10 mg of individual biogenic amines per one kg of meat. The highest levels were estimated in fat pork meat and the lowest in poultry meat from chicken breasts. These values are consistent with literature data: in fresh pork and in beef the biogenic amines levels are usually under 10 mg x kg $^{-1}$ (Rogowski, Dőhla, 1984, Pötzelberger et al., 1997). According to Silva and Glória (2002), in fresh poultry meat stored in the refrigerator at 4 °C only SPD and spermine (SPM) were detected, whilst the other amines started to appear in the chicken breasts on day 15 (in the chicken legs they started to appear earlier).

In samples of fresh chicken breast we found also only low levels of spermidine and tyramine only in traces on day 8 of storage in refrigerator in our previously performed studies (Dičáková et al., 1999). In comparison with red meat, in the samples of fresh pork and also of hoofed game meat stored at 4 °C, the increase of biogenic amine concentrations was detected on day 7, day 3 respectively (Dičáková, 2003).

CONCLUSION

The estimation of biogenic amine levels is useful for the evaluation of meat quality because increased levels of biogenic amines provide an information about microbial metabolic activities in meat before their detection by sensory analysis. Especially the increased concentrations of putrescine and cadaverine help to point out meat decomposition before it can be perceived organoleptically (Pötzelberger, 1997).

For the production of thermally untreated meat products, low initial levels of biogenic amines are of utmost importance because later, during fermentation, some amines (tyramine and histamine) can reach levels harmful for sensitive consumers.

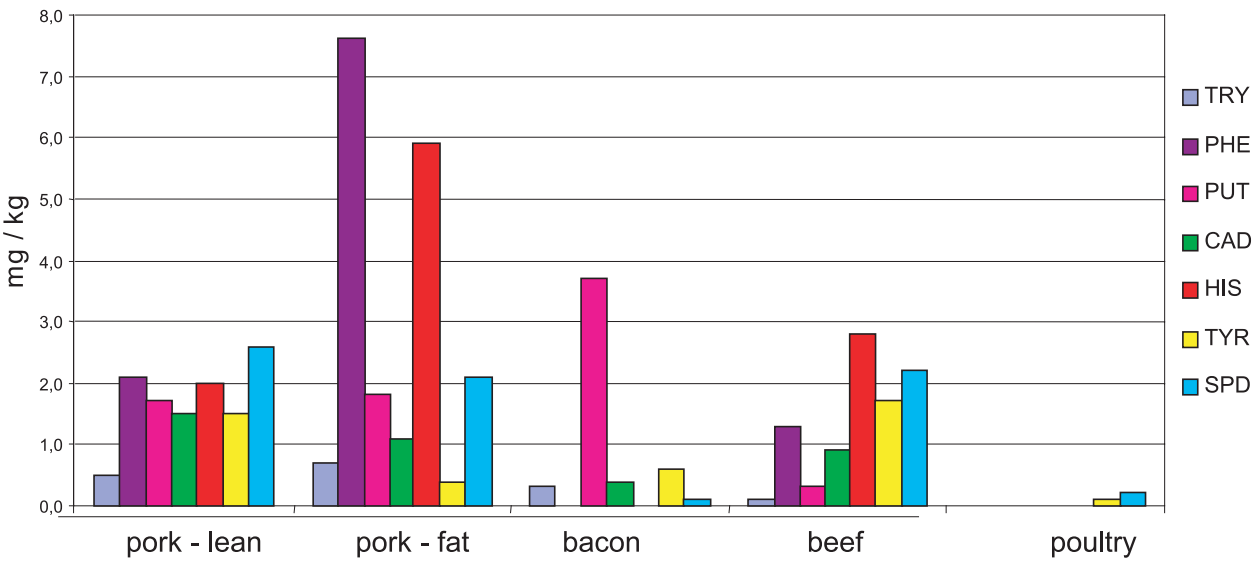
Therefore, it is important to develop and use rapid and accurate detection method to estimate biogenic amine levels in meat and meat products. The above-described method proved to be accurate and sensitive enough for routine use.

SAŽETAK

PROCJENA BIOGENIH AMINA U MESU POMOĆU HPLC METODE

HPLC metoda je upotrebljena za procjenu razina biogenih amina triptamina (TRY), feniletilamina (PHE),

▼ **Fig 2.** Levels of individual biogenic amines in different groups of meat samples (mg x kg $^{-1}$)
Razine pojedinih biogenih amina u raznim grupama uzoraka mesa (mg x kg $^{-1}$)



putrescina (PUT), kadaverina (CAD), histamina (HIS), tiramina (TYR) i spermidina (SPD) u mesu. Uzeti su uzorci mesa koje se koristi u proizvodnji fermentiranih mesnih proizvoda.

Također su uzeti uzorci svježih pilećih prsa pohranjenih na sobnoj temperaturi od 4 °C koji su analizirani drugog dana pohrane. Dobiveni rezultati kretali su se od 0 do 20.0 mg x kg^{-1} i bili su sukladni podacima iz literature. Razine biogenih amina u pilećem mesu su bile niže.

Opisana metoda je brza, precizna i prikladna za procjenu razina biogenih amina u mesu.

***Ključne riječi:** biogeni amini, meso, HPLC metoda*

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ZNAČENJE KEMIJSKIH ANALIZA U OCJENI SASTAVA I ZDRAVSTVENE ISPRAVNOSTI NAMIRNICA ANIMALNOG PODRIJETLA

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SAŽETAK

U analitici namirnica, od određivanja osnovnog kemijskog sastava, nutritivnih tvari, pa sve do kontaminanata, koriste se brojni analitički postupci. Dobiveni podaci mogu se koristiti u različite svrhe, od temeljnih znanstvenih istraživanja, pa do potreba monitoringa, o čemu također ovisi odabir analitičkog postupka. Svakako treba imati na umu da su osnovni zahtjevi za bilo koju metodu selektivnost, točnost, preciznost i ponovljivost.

UVOD

Analitička je kemija znanost prikupljanja, određivanja i tumačenja informacija o materijalnom sustavu uz pomoć znanstvenih metoda. Analitika je znanstvena disciplina, ali i umjetnost i zanat jer je potrebna i kreativnost i eksperimentalno iskustvo za postizanje rezultata. U središtu analitičkih razmatranja stoji uvijek uzorak, koji predstavlja sredinu iz koje je izdvojen. Važno je da se uzorak ne mijenja,

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