Journal of Microbiology,
Biotechnology and
Food Sciences

Buňka et al. 2012 : 2 (1) 209-218

REGULAR ARTICLE

BIOGENIC AMINES CONTENT IN SELECTED COMMERCIAL FERMENTED PRODUCTS OF ANIMAL ORIGIN

František Buňka[*1], Ludmila Zálešáková[1], Radka Flasarová[1], Vendula Pachlová[1], Pavel Budinský[2], Leona Buňková[3]*

Address: Assoc. Prof. František Buňka, Ph.D., Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata University in Zlin, nám. T. G. Masaryka 5555, 760 01 Zlín, Czech Republic; tel.: +420 576 033 011,

[1] Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata University in Zlin

[2] Faculty Hospital in Motol, V Úvalu 84, 15006 Prague,

[3] Department of Environmental Protection Engineering, Faculty of Technology, Tomas Bata University in Zlin

*Corresponding author: bunka@ft.utb.cz

ABSTRACT

The aim of this study was to monitor of biogenic amines contents in commercial fermented products, especially various type of ripening cheeses and fermented meat products (15 cheese samples and nine dry fermented meat products obtained from Czech Republic, Slovak Republic, Poland, and Germany). Furthermore, the changes in samples during storage were also observed. The samples were stored at 6±1°C. The samples were taken the first day of storage and the last day of shelf-life. The biogenic amines content was determined using ion-exchange liquid chromatography with post-column derivatization and photometric detection. The content of biogenic amines increased during storage. The highest biogenic amines content at the end of storage was observed in Dutch-type cheeses and in Smear cheeses, in both the concentrations exceeded 1 000 mg.kg^{-1}. Latter mentioned amount may have serious negative impact on consumer health. The lowest concentration of biogenic amines was detected in Blue cheese.

Keywords: biogenic amines; cheese; fermented meat products; health risks;

INTRODUCTION

Biogenic amines (BA) are low molecular nitrogen compounds. They are formed by microorganism amino acid decarboxylation. Biogenic amines are naturally presented in low concentration in animals, plants and microorganism, where they can discharge many important physiological functions (hormone precursors, source of nitrogen for biochemical reaction in organism, stabilization of macromolecular membranes, etc.) (**Edwards et al., 1981; Nout, 1994; Roginski et al., 2012; Santos, 1996; Shalby, 1996)**. High concentrations of biogenic amines can be toxic for the human organism; they influence neurotransmitters, changes of perception, smooth muscle contractions, headache etc. Above mentioned symptoms could lead to dead in cases of ill persons (**Bodmer et al., 1999; Buňková et al., 2009; Halász et al., 1994; Kalač et al., 1997; Kalač et al., 2005; Komprda, 2004; Santos, 1996)**. Nowadays, it is not determined the maximum amount of biogenic amines in dairy and meat products. However, some authors present, that the amount of 8–40 mg kg^{-1} of histamine can cause the mild poisoning, 40–100 mg kg^{-1} medium poisoning, and the total BA content over 100 mg kg^{-1} could cause very serious poisoning. Tyramine amount of over than 100 mg kg^{-1} can also give indication of poisoning (for example swelling creation, etc.) (**Fox et al., 2004; Önal, 2007)**.

The processing technology can significantly influence the formation of biogenic amines and their final concentrations in sample. Biogenic amines are formed in foodstuffs by the starter and non-starter microorganisms, in fermented foodstuff mainly by occurrence of lactic acid bacteria (e. g. *Lactococcus*, *Lactobacillus* and *Enterococcus* strains). Also the contaminating microflora could result in high concentration of BA in sample. In non-pasteurized milk and in products with inappropriate hygiene conditions during the processing technology, *Enterobacteriaceae* strains could be observed which are evaluated as regular producers of BA. Higher concentrations of BA could indicate spoiling; therefore the biogenic amines can be considered as the indicators of food freshness (**Bodmer et al., 1999; Buňková et al., 2009; Edwards et al., 1981; Halász et al., 1994; Roginski et al., 2012).**

The conditions for the biogenic amines formation could be divided into direct ones (presence of a sufficient amount of free amino acid and presence of the microorganisms with decarboxylation activity); and indirect ones (conditions affecting the optimum growth of microorganisms with decarboxylation activity – presence of source of carbohydrate, suitable temperatures, pH, presence of oxygen, etc.). Certain indirect conditions are dependent on the specific microorganism species (**Komprda, 2004; Nout, 1994; Roginski et al., 2012).**

Fermented foods are products in which the conversion of the present carbohydrates into organic acids during maturation occurs. The fermentation process is realized by mainly starter microorganisms (lactic acid bacteria), which are added to products during technological process. In the fermented dairy and meat products, lactose is especially converted into lactic acid; the rest could form carbon dioxide, other organic acids, other carbon compounds etc. Proteolysis is another process that usually takes place (**Fox et al., 1998; Fox et al., 2004; Hui, 1993; Scott et al., 1998)**.

The aim of this study was to analyze the content of eight biogenic amines in commercial fermented products, especially various types of ripening cheeses and fermented meat products (cheese samples and dry fermented meat products obtained from Middle Europe area) during storage.

MATERIAL AND METHODS

Sample characterization

Fifteen cheeses samples and nine dry fermented meat products were bought in Central Europe (Czech Republic, CZ; Slovak Republic, SK; Poland, PL; and Germany, D). Cheeses were divided into 5 groups – surface-mould cheese (Camembert-type cheese; SMC), blue-cheese (BC), double-mould cheese (DMC), Dutch-type cheese (DTC) and smear cheese (SC). Dry fermented meat products (DFMP) were the last group. Six samples (from the same batch) were purchased. The samples were analyzed in the first day of storage (the day when the sample was obtained; 3 samples from a batch) and the last day shelf-life (3 samples from a batch). Products were stored at 6±1°C (the storage period of each sample is presented in Table 1).

The samples were lyophilized by using the Alpha 1–4 LSC (Christ, Germany, Osterode am Harz), the temperature –40 °C and the pressure ~12 Pa at the day of sampling.

Biogenic amines extraction

Extracts were prepared from the lyophilized samples according to **Buňková et al. (2010)**. The mixture of 1 g lyophilized sample with 5 ml sodium-citrate buffer (pH 2,2) was homogenized for 45 minutes at the room temperature and then it was centrifuged by EA 21 (Hettich Zentrifuge, Germany, Tuttlingen) at 6 000 *g* for 20 minutes. The supernatant was strained into 10-ml volumetric flask and the sediment was reextracted again with 5 ml sodium-citrate buffer (pH 2,2) as described above. Both supernatants were mixed in 10-ml volumetric flask and filled up to the mark. The supernatant was filtered and centrifuged at 15 000 *g* for 45 minutes in 4 °C using Mikro 200R (Hettich Zentrifuge, Geraminy, Tuttlingen). Centrifuged samples were filtered through 0.22 µm nylon filter. Each extraction was repeated for three times (we obtained 9 extracts from the sample: 3 extraction from a samples × 3 samples were used).

Chromatographic analysis

Eight biogenic amines (histamine – HIM; tyramine – TYM; 2-phenylethylamine – PHE; putrescine – PUT; cadaverine – CAD; agmatine – AGM; spermidine – SPD; and spermine – SPN) were determined by ion-exchange chromatography (column 55 x 3.7 mm, filled with ion-exchange OSTION Lg ANB; Ingos, Prague, Czech Republic) with post-column ninhydrine derivatization and photometric detection (λ = 570 nm) using Automatic amino acid analyzer AAA 400 (Ingos, Prague, Czech Republic). Chromatographic analysis was performed according to **Buňková et al. (2009).** Each extract was analyzed twice (n=18). The standards of Sigma Aldrich (St. Louis, MO, USA) for chromatographic analysis of biogenic amines were used.

RESULTS AND DISCUSSION

The obtained values of selected biogenic amines at the beginning and at the end of storage period are shown in Table 1. Spermidine, spermine and agmatine were not detected in any of the analyzed samples. Histamine, one of the most important biogenic amines, was detected in 4 groups (surface-mould cheese, blue-cheese, smear cheese and dry fermented meat products). Higher histamine concentration at the beginning of the storage time was detected in dry fermented meat products (up to 32 mg kg^{-1}). The significant increase of histamine content was observed in the group of dry fermented meat products, the values increased to the final concentration above 150 mg kg^{-1}.

Tyramine was detected in all groups except the double-mould cheeses. In the first day of storage, the tyramine concentrations were relatively low. The exceptions represented: a sample of the blue cheeses (B4), a case of Dutch-type cheeses (D3) and a sample of the smear cheeses (E2), where the tyramine amount were above 100 mg kg^{-1}. At the end of storage, the limit of 100 mg kg^{-1} was exceeded in 10 samples originated form 4 product groups (blue cheeses, Dutch-type cheeses, smear cheeses, and also dry fermented meat products).

Phenylethylamine was determined in three groups (Dutch-type cheeses, smear cheeses and dry fermented meat products) and these amounts were also relatively low (6.3–35.3 mg kg^{-1}). The most significant phenylethylamine concentrations were obtained in Dutch-type cheeses and dry fermented meat products at the end of storage.

Putrescine was detected in all analyzed groups. At the beginning of the storage, the high putrescine amounts were observed in one sample of the smear cheese group (E2) and one product of the dry fermented meat products (F7) (201.7 mg kg^{-1} and 134.3 mg kg^{-1}, respectively). At the end of the storage, the putrescine concentrations were significantly higher in comparison with amounts at the beginning of storage. Some concentrations of putrescine exceeded 200 mg kg^{-1}.

At the beginning of the storage, the most of samples showed insignificant amount of cadaverine. Only in two samples, high amount of cadaverine were observed (E2 and E3). The same situation was recorded at the end of storage. The cadaverine concentrations in samples E2 and E3 were very high (hundreds milligrams per kilogram). In the rest of samples, the amounts of cadaverine were acceptable.

Besides monitoring of individual BA contents, total amount of all BA should also be explored because of their mutual potentiation capacity. Approximately 40% of the tested samples demonstrated total level of all the monitored BA below 100 mg kg^{-1}. A worrying finding is represented by the fact that almost 60% of products (after storage period) contained more than 100 mg kg^{-1} of BA and that 35% of the samples even included more than 1 000 mg kg^{-1} of BA.

Also in the dry fermented meat products (F1 – F9) was monitored the total BA content at the end of storage time. In 3 samples from dry fermented meat products was the total BA amount bellow 100 mg kg^{-1}. Nevertheless the total amount of BA exceeding the limit 100 mg kg^{-1} was detected in 6 samples of dry fermented meat products and even in 3 samples this amount exceeded 300 mg kg^{-1}.

Table 1 The biogenic amines concentration at the beginning (B) and the end (E) of the storage and the duration of storage period (days) at 6°C

Group	Sample	Time *	Storage (days)	Biogenic amines content [mg kg^{-1}]				
				HIM [1)]	TYM [2)]	PEA [3)]	PUT [4)]	CAD [5)]
SMC[a)]	A1	B		ND**	ND	ND	5.0 ± 0.1	ND
		E	18	ND	ND	ND	11.0 ± 0.5	ND
	A2	B		12.4 ± 0.6	18.8 ± 1.1	ND	2.6 ± 0.1	20.1 ± 0.7
		E	22	35.4 ± 1.5	93.0 ± 3.8	ND	6.9 ± 0.4	31.0 ± 0.5
	A3	B		ND	ND	ND	6.5 ± 0.3	ND
		E	18	ND	ND	ND	2.9 ± 0.1	ND
BC[b)]	B1	B		ND	ND	ND	2.0 ± 0.1	ND
		E	14	ND	ND	ND	6.3 ± 0.3	ND
	B2	B		ND	ND	ND	ND	ND
		E	20	ND	15.4 ± 0.4	ND	2.0 ± 0.1	ND
	B3	B		4.9 ± 0.2	10.7 ± 0.5	ND	10.5 ± 0.4	ND
		E	19	7.4 ± 0.3	140.6 ± 5.8	ND	30.4 ± 0.7	3.0 ± 0.1
	B4	B		3.2 ± 0.1	142.9 ± 6.0	ND	14.8 ± 0.6	2.3 ± 0.1
		E	17	6.0 ± 0.1	1 069.6 ± 9.6	ND	81.2 ± 0.5	10.2 ± 0.1
DMC[c)]	C1	B		ND	ND	ND	1.8 ± 0.1	ND
		E	18	ND	ND	ND	5.6 ± 0.1	ND
DTC[d)]	D1	B		ND	54,0 ± 0.1	ND	ND	ND
		E	90	ND	1 015.0 ± 20.1	74.2 ± 0.7	11.2 ± 0.1	ND
	D2	B		ND	25.2 ± 1.1	35.3 ± 1.4	ND	ND
		E	64	ND	973.5 ± 19.5	163.5 ± 1.3	108.9 ± 0.7	67.0 ± 0.4
	D3	B		ND	365.1 ± 8.2	ND	37.4 ± 1.3	ND
		E	87	ND	991.5 ± 19.6	140.0 ± 1.7	459.8 ± 13.0	ND
SC[e)]	E1	B		ND	ND	ND	ND	ND
		E	19	ND	ND	ND	ND	ND
	E2	B		2.8 ± 0.1	167.9 ± 5.0	28.9 ± 0.1	201.7 ± 7.2	630.6 ± 21.1
		E	19	5.0 ± 0.1	243.9 ± 0.5	59.7 ± 1.3	272.3 ± 10.0	1 428.4 ± 46.8
	E3	B		ND	34.5 ± 2.5	6.3 ± 0.2	7.3 ± 0.4	170.6 ± 5.5
		E	19	ND	77.7 ± 2.8	12.1 ± 0.2	15.2 ± 0.3	245.7 ± 6.0
DFMP[f)]	F1	B		ND	12.4 ± 0.6	6.4 ± 0.2	ND	ND
		E	70	ND	181.7 ± 8.3	14.1 ± 0.6	ND	ND
	F2	B		ND	54.0 ± 1.0	ND	ND	ND
		E	36	ND	148.5 ± 6.9	24.1 ± 1.1	ND	ND
	F3	B		ND	ND	ND	ND	ND
		E	24	ND	ND	ND	ND	ND
	F4	B		ND	42.3 ± 0.6	24.7 ± 0.3	ND	ND
		E	70	ND	228.1 ± 7.9	42.7 ± 1.0	ND	ND
	F5	B		31.7 ± 1.2	35.0 ± 1.7	ND	15.1 ± 0.8	6.3 ± 0.4
		E	62	151.8 ± 3.5	195.0 ± 4.5	ND	31.7 ± 1.4	16.8 ± 0.2
	F6	B		ND	3.6 ± 0.2	ND	3.1 ± 0.2	ND
		E	70	ND	15.3 ± 0.5	ND	18.1 ± 0.6	ND
	F7	B		12.9 ± 0.5	81.0 ± 2.5	ND	134.3 ± 4.8	ND
		E	58	29.6 ± 1.4	86.6 ± 1.3	ND	225.1 ± 4.6	ND
	F8	B		ND	17.5 ± 0.4	ND	ND	ND
		E	63	ND	46.4 ± 0.5	ND	ND	ND
	F9	B		2.4 ± 0.1	26.7 ± 0.6	ND	57.1 ± 0.7	4.1 ± 0.1
		E	46	73.1 ± 1.7	90.4 ± 2.7	ND	210.4 ± 3.3	15.0 ± 0.2

[1)] Histamine, [2)] Tyramine, [3)] Phenylethylamine, [4)] Putrescine, [5)] Cadaverine
[a)] surface-mould cheese, [b)] blue cheese, [c)] double-mould cheese, [d)] Dutch-type cheese, [e)] smear cheese, [f)] dry fermented meat products
* ND – not detected
** B – the beginning of storage; E – the end of storage

The highest total biogenic amounts were detected in smear cheese samples. It was detected in one sample of this group over 2 000 mg kg^{-1}. In smear cheeses were detected mainly the high concentrations of cadavarine (almost 1 500 mg kg^{-1}). **Standardová et al. (2008)** presented the similar results in smear cheeses, especially higher amount of cadaverine (1 100 mg kg^{-1}). The second group with the highest total biogenic amount were Dutch-type cheeses. These values reached 1 000 mg kg^{-1} in all analyzed samples of this group. These higher concentrations are rather sporadic. In other publications are the total biogenic amounts lower, e.g. **Leuschner et al. (1998)** presented twice lower detected values in Dutch-type cheese (~500 mg kg^{-1}), **Komprda et al. (2007)** shows more lower total biogenic amounts (20–400 mg kg^{-1}). In other analyzed samples (mould cheeses, blue cheeses, double mould cheeses) weren´t the total biogenic amounts higher than 400 mg kg^{-1}. Only in one sample of blue cheeses was the biogenic amines value over 1 000 mg kg^{-1}. The same results were presented by **Mayer et al. (2010), Standarová et al. (2008), Standarová et al. (2009)**. In dry fermented meat products the total biogenic amount reached almost 400 mg kg^{-1}. Similar amounts are shown by **Suzzi and Gardini (2003).**

Detected biogenic amines values can be influenced by many factors – concrete cheese type, conditions and procedure of production, quality of starting raw materials, storage conditions, etc. It was detected that in 25 % of all analyzed samples the total biogenic amount reached the limit 900 mg kg^{-1}. **Ancín-Azpilicueta et al. (2008)** shows that this value can lead to serious health problems and it can cause health risks for consumers such foodstuff.

CONCLUSION

Altogether 14 samples of dairy products and 9 samples of dry fermented products were analyzed for the biogenic amines presence. These samples were produced in Central Europe. The histamine and phenylethylamine concentration were very low at the beginning of the storage time, but concentrations of tyramine, putrescine a cadaverine exceeded the limit 100 mg kg^{-1} in sporadic samples. During the storage time the amounts of individual BA increased. Histamine and phenylethylamine amounts were almost insignificant. Tyramine, putrescine and cadaverine concentrations were in most dairy and dry fermented meat products approximately 100 mg kg^{-1} at the end of storage time. And in some samples was this limit many times exceeded. In 35% of dairy products and in 6 samples of dry fermented meat products was detected the total biogenic amines amount over 100 mg kg^{-1}. This amount can be considered critical from the toxicological point. These detected biogenic amines values point to the problem of the formation of high biogenic amines amount in dairy and dry fermented meat products.

Acknowledgement: This study was supported by projects of the internal grant of Tomas Bata University in Zlín No. IGA/FT/2012/027 funded from the resources for specific university research and GACR 503/11/1417.

REFERENCES

ANCÍN-AZPILICUETA C. – GONZÁLEZ-MARCO A. – JIMÉNEZ-MORENO N. 2008. Current knowledge about the presence of amines in wine. In *Critical Reviews in Food Science and Nutrition*, Vol. 48, No. 3, p. 257–275.

BODMER S. – IMARK C. – KNEUBÜHL M. 1999. Biogenic amines in foods: Histamine and food processing. In *Inflammation Research*, Vol. 48, No. 6, p. 296-300.

BUŇKOVÁ L. – BUŇKA F. – HLOBILOVÁ M. – VAŇÁTKOVÁ Z. – NOVÁKOVÁ D. – DRÁB V. 2009. Tyramine production of technological important strains of *Lactobacillus, Lactococcus* and *Streptococcus.* In *European Food Research and Technology*, Vol. 229, No. 3, p. 533-538.

BUŇKOVÁ L. – BUŇKA F. – MANTLOVÁ G. – ČABLOVÁ A. – SEDLÁČEK L. – ŠVEC P. et.al. 2010. The effect of ripening and storage conditions on the distrinution of tyramine, putrescine and cadaverine in Edam-cheese. In *Food Microbiology,* Vol. 27, No. 7, p. 880-888.

EDWARDS S. T. – SANDINE W. E. 1981. Symposium: Microbial metabolites of importance in dairy products. In *Journal of Dairy Science,* Vol. 64, No. 12, p. 2341-2438.

FOX. P. F. – MCSWEENEY P. 1998. Dairy chemistry and biochemistry, 1st edition, Tullamore: Thomson science, 478 p., ISBN 0-412-72000-0.

FOX. P. F. – MCSWEENEY P. – COGAN T. M. – GUINEE T. P. 2004. Cheese chemistry, Physics and Microbiology, Major cheese groups, volume 2, 3rd edition, London: Elsevier Academic Press, 577 p., ISBN 9-780-41258-2301.

HALÁSZ A. – BARÁTH Á. – SIMON-SARKADI L. – HOLZSPAFEL W. 1994. Biogenic amines and their production by microorganism in food. In *Trends in Food Science & Technology*, Vol. 5, No. 2, p. 42-49.

HUI H. Y. 1993. Dairy science and technology handbook, New York: Wiley-VCH, 385 p., ISBN 1-56081-078-5

KALAČ P. – HLAVATÁ V. – KŘÍŽEK M. 1997. Concentrations of five biogenic amines in Czech beers and factors affecting their formativ. In *Food Chemistry*, Vol. 58, No. 3, p 209-214.

KALAČ P. – KŘÍŽEK M. 2005. Biogenní aminy a polyaminy v potravinách a jejich vliv na lidské zdraví. In *Potravinářská Revue*, Vol. 2, p. 40-42.

KOMPRDA T. 2004. Obecná hygiena potravin. Brno: Mendelova zemědělská a lesnická univerzita, 145 p., ISBN 978-80-7157-757-7.

KOMPRDA T. – SMĚLÁ D. – NOVICKÁ K. – KALHOTKA L. – ŠUSTOVÁ K. – PECHOVA P. 2007. Content and distribution of biogenic amines in Dutch-type hard cheese. In *Food Chemistry*, Vol. 102, No. 1, p. 129–137.

LEUSCHNER R. G. K. – KURIHARA R. – HAMMES W. P. 1998. Effect of enhanced proteolysis on formation of biogenic amines by lactobacilli during Gouda cheese ripening, In *International Journal of Food Microbiology*, Vol. 44, No. 1–2, p. 15–20.

MAYER H. K. – FIECHTER G. – FISCHER E. 2010. A new ultra-pressure liquid chromatography method for the determination of biogenic amines in cheese. In *Journal of Chromatography A,* Vol. 1217, No. 1, p. 3251–3257.

NOUT M. J. R. 1994. Fermented foods and food safety. In *Food Research International*, Vol. 27, No. 3, p. 291-298.

ÖNAL A. 2007. A review: current analytical methods for determination of biogenic amines in food. In *Food chemistry,* Vol. 103, No. 4, p. 1475-1486.

ROGINSKI R. – FUQUAY J. W. – FOX P. F. 2012. Encyclopedia of dairy science, London: Academic press, 2799 p., ISBN 0-12-227235-8.

SANTOS M. H. S. 1996. Biogenic amines: their importance in fous. In *Journal of Food Microbiology*, Vol. 29, No. 2-3, p. 213-231.

SCOTT R. – ROBINSON R. K. R. – WILBEY R. A. 1998. Cheesemaking practice, Verlag: Springer, 449 p., ISBN 978-07514-0417-3.

SHALBY A. R. 1996. Significance of biogenic amines to food safety and human health. In *Food Research International*, Vol. 29, No. 7, p. 675-690.

STANDAROVÁ E. – BORKOVCOVÁ I. – VORLOVÁ L. 2008. Obsah biogenních aminů v sýrech z české obchodní sítě. In *Veterinářství*, Vol. 58, No. 1, p. 735 – 739.

STANDAROVÁ E. – VORLOVÁ L. – BORKOVCOVÁ I. 2009. Zastoupení vybraných biogenních aminů v sýrech s bílou plísní na povrchu. In *Acta fytotechnica et zootechnica* – mimoriadne číslo, p. 610-617.

SUZZI G. – GARDINI F. 2003. Biogenic amines in dry fermented sausages: a review, In *International Journal of Food Microbiology*, Vol. 88, No. 1, p. 41– 54.