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BIOGENIC AMINES IN SMEAR AND MOULD-RIPENED CHEESES

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ABSTRACT

The aim of the study was the monitoring of six biogenic amines (histamine, tyramine, phenylethylamine, tryptamine, putrescine, and cadaverine) and two polyamines (spermidine and spermine) in 30 samples of dairy products purchased in the Czech Republic, namely in 15 samples of mould-ripened cheeses and in 15 samples of smear-ripened cheeses. A further goal was the microbiological analysis of the individual samples of cheeses (total count of microorganisms, number of enterobacteria, enterococci, lactic acid bacteria, yeasts and moulds). The monitored biogenic amines were analyzed by a high performance liquid chromatography equipped with a UV/VIS DAD detector. The amount of enterobacteria in fresh cheese exceeded 10^5 CFU.g^{-1}. In smear-ripened cheese flavourless (Romadur type), the amount was >10^3 CFU.g^{-1} and 10^4-10^5 CFU.g^{-1} in smear-ripened cheese with flavour. Biogenic amines were observed in two groups of blue cheeses (white veined cheese and blue veined cheese) and smear-ripened cheeses. In both groups, there is a possibility of the presence of biogenic amines because the number of microorganisms and concentration of free amino acids increase during ripening. In ten samples of soft smear-ripening acid cheese and in smear-ripened cheese, the total content of biogenic amines were 22-1000 mg.kg^{-1} and in 5 samples of these cheeses, it was in range 1000-6000 mg.kg^{-1}. The total amount of biogenic amines in the blue cheeses were in range 40-600 mg.kg^{-1}. The presense of the tyramine was observed in the all analysed cheeses. The tyramine producing strains generated more than 900 mg.kg^{-1} of this biogenic amine. The production of tryptamine in the analysed cheeses was not proved by this study. The results of this study show that biogenic amines and polyamines are common in cheese. However, in some cases, they can pose a significant health danger for consumers. Any legislative control authority does not monitor them, as they are secondary metabolites even though they are potential health risks.

Keywords: biogenic amines; high performance liquid chromatography; smear-ripened cheese; mould-ripened cheese

INTRODUCTION

Biogenic amines (cadaverine, histamine, phenylethylamine, putrescine, tyramine, spermidine, spermine; BA) are formed by microorganisms demonstrating decarboxylase activity from corresponding amino acids. Amination and transamination of aldehydes and ketones is also a minor way of creating BA. BAs are present in all foods as products of protein metabolism. They are also present in fermented products, such as smear and mould-ripened cheeses in higher amount **(Loizzo et al., 2012)**. Lack of hygiene or influence of contaminating microflora during cheese ripening can lead to BA formation. Great amount of free amino acids (precursors of BAs) is formed by proteolysis of caseins. Some bacteria species (family *Enterobacteriaceae* or strains of *Lactobacillus*, *Streptococcus*, *Micrococcus*, *Enterococcus* and *Pseudomonas*) are able to produce decarboxylases. Tyramine, histamine, putrescine, cadaverine and tryptamine are usually detected in cheeses **(Silla Santos, 1996; Shalaby, 1996)**. Intensity of decarboxylase reactions depends on the presence of microorganisms (respectively on the presence of appropriate species). Concentration of free amino acids and various environmental factors (for example pH values, oxygen and carbon availability, temperature, the amount of amino acids, water activity can affected the decarboxylase activity **(Halász et al., 1994; Bardocz, 1995)**. BAs are related to alimentary poisoning and they can endanger consumers´ health **(Silla Santos, 1996)**. Tyramine and histamine can affect vascular and central neural system. Putrescine and cadaverine support toxicity of other biogenic amines **(Mayer et al., 2010)**. Amount of BAs in cheeses is influenced by various factors. Main factors during the cheese manufacture are ripening period and storage. Storage of cheese in higher temperatures leads to the increase of BAs. Authors **Mayer et al., (2010)** and **Pachlová et al., (2013)** reported, that the same amounts of BAs were detected in cheese ripened at 5 °C for three weeks and at 20 °C for one week. BA formation can be also influenced by high-pressure treatment during ripening. There can be increased production of peptides and amino acids, which can support of BA production **(Novella-Radríguez et al., 2002)**.

Higher amount of BAs in smear-ripened cheese can be affected by starter or non-starter bacteria (contaminating microflora) during milk fermentation (enterobacteria, enterococci, *Flavobacterium* spp., *Microbacterium* spp., *Bacillus* spp., actinobacteria, etc.). Undesirable pathogens, for example *Listeria monocytogenes*, *Staphylococcus aureus* and pathogenic strains of *Escherichia coli*, can be present in smear-ripened cheese too, due their favourable conditions for growth of this bacteria **(Monnet et al.,**

2010). BA amount is significant in these cheeses because they contain higher amount of decarboxylase-positive bacteria. The strains of *Lactobacillus* produce histamine and putrescine. Some strains, for example *Lactobacillus brevis*, can also create tyramine. Enterococci are important members of microflora in smear-ripened cheese. These bacteria can create tyramine by tyrosine decarboxylation because of its resistance against high temperature and higher NaCl concentration. Significant relation between bacteria species and biogenic amines concentration was observed, for example enterococci and production of phenylethylamine, lactococci and cadaverine and tyramine. In smear-ripened cheeses, there is mainly cadaverine, tyramine, histamine and putrescine detected **(Loizzo et al., 2012)**.

MATERIAL AND METHODOLOGY

Biogenic amines were determined in 30 samples of cheese. There were 9 samples of white veined cheese and 6 samples of blue veined cheese. These products are natural, with no flavour added. The samples of soft smear-ripening acid cheese, soft smear-ripening acid cheese smoked, soft smear-ripening acid cheese flavoured pepper and flavoured garlic, soft cold-ripened cheese unflavoured, flavoured green pepper and flavoured walnuts, smear-ripened cheese flavoured garlic and herbs, flavoured chilli and further 6 samples od smear-ripened cheese were analyzed. All the cheeses were purchased in stores in Czech Republic. The cheeses were stored at temperature 18 °C at least. For each cheese sample, there was identified the total number of aerobic and facultative anaerobic mesophilic microorganisms on Plate Count Agar (PCA), enterobacteria on Endo Agar, enterococci on Slanetz Bartley Agar, lactic cocci on M17 Agar, lactic rods on MRS Agar and yeast and mould on Chloramphenicol Yeast Glucose Agar (CHYGA). Plates with microorganisms were cultivated at temperature 30 °C for 48 hours, plates with Endo Agar at 37 °C for 24 hours and plates with CHYGA at temperature 20 °C for 5 days. Grown colonies were evaluated and converted to $CFU.g^{-1}$ after cultivation.

The production of seven biogenic amines (cadaverine, CAD; histamine, HIS; phenylethylamine, PHE; putrescine, PUT; tyramine, TYR; spermidine, SPD; spermine, SPN) was monitored by an high performance liquid chromatography system equipped with a binary pump; an autosampler (LabAlliance, USA); a column thermostat; a UV/VIS DAD detector (λ = 254 nm); and a degasser (1260 Infinity, Agilent Technologies, USA).

10 ml (c = 0.6 $mol.L^{-1}$) perchloric acid (Sigma) was added to 1 g of lyophylized cheese sample. The sample was mixed and shaken for 30 minutes, centrifuged (4000 x g) and then an upper part was cast. 7 mL (c = 0.6 $mol.L^{-1}$) perchloric acid was added to the sediment. This process was repeated 2 times. Then, the mixture was filtered (porosity 0.45 µm) and the filtrate was derivatized with dansyl chloride according to **Dadáková et al., (2009)**. 1.7-heptandiamine was used as internal standard. The derivatized samples were filtered (porosity 0.22 µm) and applied to a column (Cogent HPLC Column HPS C18, 150 x 4.6 mm, 5 µm). Terms of the separation of the observed biogenic amines are described in works of **Buňková et al., (2013)**.

RESULTS AND DISCUSSION

In Czech standard ČSN 56 9606, there is no limit for the total amount of microorganisms in smear-ripened cheese or in blue cheese. The limit is probably not listed because various doses of the culture of microflora is intentionally added to these cheeses depending on the type of the cheese and microorganisms (MO), which affects the aroma significantly, grow there during the ripening **(Buňková et al., 2012)**. Also, the standards often focus on determining microorganisms causing diseases in human, or microorganisms able to devalue the final product. The presence of such microorganisms is usually not expected in cheese (or they are present in very low numbers) because microorganisms in the starter culture are supposed to overgrow them. Identified amounts of MO might include mesophilic starter culture of the genera *Lactococcus* and *Leuconostoc*, bacteria *Brevibacterium linens*, coryneform bacteria, staphylococci and yeast **(Görner et al., 2004)**. On the other hand, many lactic acid bacteria, like coryneform bacteria, are more difficult to cultivate and that is why they are not detected on PCA. This might lead to the conclusion that only microorganisms without specific cultivation requirements can be identified on PCA and thus they can be classified among contaminating microflora (without causing any significant changes in the final product).

Soft smear-ripening acid cheese

In tested samples of soft smear-ripening acid cheese, the total amount of microorganisms (CFU) was defined to be between 10^5-10^9 $CFU.g^{-1}$. Most of microorganisms were determined in samples of soft smear-ripening acid cheese unflavoured, followed by flavoured cheeses and the least of MO was present in samples of cold-ripened cheese. Numbers of enterobacteria were always over 10^5 $CFU.g^{-1}$ in soft smear-ripening acid cheese, >10^3 $CFU.g^{-1}$ in samples of smear cold-ripened cheese unflavoured and 10^4-10^5 $CFU.g^{-1}$ in flavoured cheeses. Lactic acid bacteria were present in cheese in amounts ranging from 10^6-10^8 $CFU.g^{-1}$ mostly, in samples of soft smear-ripening acid cheese (10^7-10^9 $CFU.g^{-1}$). According to **Bockelmann, (2002)**, the number of lactic acid bacteria ranged between 10^2-10^7 $CFU.g^{-1}$ depending on the cheese sample. Further they state that the amount of lactic acid bacteria exceeded 10^7 $CFU.g^{-1}$ in some of the cheese samples. Similar results were achieved in this study. Enterococci were detected in 10 cheese samples with their numbers ranging from 10^3-10^6 $CFU.g^{-1}$. Most of the enterococci was found in soft smear-ripening acid cheese flavoured with its amount over >10^5 $CFU.g^{-1}$. In samples of cheese type like Limburger and Romadur unflavoured, there was a high amount of enterococci determined >10^3 $CFU.g^{-1}$, which corresponds with the studies of **ockelmann et al., (2005)** where presence of enterococci in Limburger and Romadur cheeses was set to be in an amount of >10^4 $CFU.g^{-1}$. Lactic acid bacteria form a very large group of microaerophilic to facultative anaerobic gram-positive cocci and rods fermenting saccharides to produce lactic acid. There are included genera *Lactococcus*, *Streptococcus*,

Table 1 Contend of biogenic amines in mg.kg^{-1} in tested smear-ripened cheese.

	Biogenic amines [mg.kg^{-1}]						
Samples of cheese	**Phenylethylamine**	**Putrescine**	**Cadaverine**	**Histamine**	**Tyramine**	**Spermidine**	**Spermine**
Soft smear-ripening acid cheese	11.6 ±0.8	2408.5 ±53.4	1816 ±45.9	373.8 ±11.9	1398 ±76.9	26.1 ±0.2	29.7 ±1.3
Soft smear-ripening acid cheese smoked	ND	701.2 ±14.4	1518 ±18.1	220.9 ±16.8	1118.9 ±78.3	33.3 ±1.7	38.1 ±3.2
Soft smear-ripening acid cheese flavoured pepper	ND	108.1 ±8.2	594.4 ±49.0	396.1 ±28.2	243.4 ±21.6	26.4 ±1.1	33.6 ±2.2
Soft smear-ripening acid cheese flavoured garlic	ND	407.1 ±13.3	773.8 ±35.5	302.22 ±22.1	554.8 ±17.8	31.7 ±1.0	55.7 ±1.9
Soft ripened cold cheese unflavoured	ND	ND	ND	ND	13.9 ±1.0	ND	17.2 ±1.3
Soft ripened cold cheese flavoured green pepper	ND	ND	ND	ND	12.6 ±0.4	ND	16.8 ±1.3
Soft ripened cold cheese flavoured walnuts	ND	ND	ND	ND	7.8 ±0.7	ND	14.9 ±0.4
Smear-ripened cheese flavoured garlic and herbs	ND	352.7 ±11.1	ND	ND	345.8 ±11.1	ND	32.3 ±0.7
Smear-ripened cheese flavoured chilli	ND	148.53 ±11.4	3.9 ±0.1	4.4 ±0.2	167.4 ±10.2	4.4 ±0.2	37.2 ±2.3
Smear-ripened cheese	ND	18.7 ±1.3	ND	34.1 ±0.8	38.0 ±1.0	3.0 ±0.1	57.7 ±0.7
	ND	ND	ND	ND	51.0 ±4.5	3.8 ±0.3	95.4 ±4.8
	ND	ND	4.5 ±0.3	ND	60.4 ±2.8	ND	58.4 ±3.3
	ND	ND	ND	ND	16.3 ±0.9	8.4 ±0.3	42.6 ±1.6
	ND	ND	ND	ND	44.4 ±1.2	6.4 ±0.2	85.1 ±1.9
	254.8 ±14.6	ND	2389.2 ±108.9	96.7 ±4.5	920.7 ±24.8	ND	60.2 ±4.0

* Tryptamin ND.

Enterococcus, Pediococcus, Leuconostoc and *Lactobacillus*. The amount of lactic acid bacteria exceeded 10^7 CFU.g^{-1} in some cheese samples. Similar results were acquired in this study as well. A great amount of yeast can be found in smear-ripened cheese because it is a kind of MO that gives typical aroma and taste to the cheese. Amount of yeast and mould in samples ranged in 10^4-10^6 CFU.g^{-1}.

Biogenic amines in soft smear-ripening acid cheese and in smear-ripened cheese

In ten samples of smear-ripened cheese, the total content of BA was 22-1000 mg.kg^{-1} and in 5 samples of these cheeses it was in range of 1000-6000 mg.kg^{-1}. **Standarová et al., (2010)** states in their work that values for cheese stored at 20 °C for 6 weeks are >4000 mg.kg^{-1}. Concluding from our research, the raised amount of BA in five submitted samples could be caused by improper storage of the cheese in stores. Tyramine, spermine, spermidine, putrescine, cadaverine, histamine and phenylethylamine were detected in group of smear-ripened cheeses. Putrescine was found in seven samples in the maximal amount >2000 mg.kg^{-1}. According to **Loizzo et al., (2012)**, the amount of putrescine was 532,2 mg.kg^{-1}. **Standarová et al., (2010)** claim that the putrescine amount is 212 mg.kg^{-1} when stored at 5 °C during 4 weeks. Cadaverine was detected in seven cheese samples as well. Its numbers ranged from 3-2400 mg.kg^{-1}. **Loizzo et al., (2012)** presented that the amount of cadaverine is >700 mg.kg^{-1}. According to **Standarová et al., (2010)** the cadaverine amount in this sort of cheese stored at 5 °C for 4 weeks was >400 mg.kg^{-1}. Tyramine, along with spermine, was detected in all analyzed samples of cheeses. It was present in rather wide range 8-1398 mg.kg^{-1}. **Mayer et al., (2010); Loizzo et al., (2012); Standarová et al., (2010)** detected tyramine in the same intervals. The amount of histamine in the studied samples of cheeses was 4-400 mg.kg^{-1}. This BA was found in seven samples. **Loizzo et al., (2012)** present the amount to be 168.3 mg.kg^{-1}, **Standarová et al., (2010)** stated 216 mg.kg^{-1} of histamine in soft smear-ripening acid cheese and the values goes up to 500 mg.kg^{-1} in the study of **Mayer et al., (2010)**. Spermidine was found in 9 out of 15 samples of analyzed cheeses. Compared to the other biogenic amines, its amount in the samples was rather low.

Mould cheese

Groups of determined microorganisms in samples of blue cheese were same like in the samples of smear-ripened cheese. The total number of microorganisms in all of the observed samples was 10^5-10^7 CFU.g^{-1}. Enterobacteria was detected only in sample of white veined cheese (dry matter 28% w/w, fdm-fat in the dry matter 49.5% w/w) with its amount being 5.7 x 10^3 CFU.g^{-1}. The amount of enterococci was determined only in sample of white

Table 2 Content of biogenic amines in $mg.kg^{-1}$ in tested mould-ripened cheeses.

Characterisation of samples of cheese			Concentration of biogenic amines [$mg.kg^{-1}$]				
	dry matter (% w/w)	fdm (% w/w)	Putrescine	Cadaverine	Tyramine	Spermidine	Spermine
White veined cheese	26.4	48	15.8 ±1.2	ND	20.0 ±0.8	ND	38.3 ±1.1
	51	60	20.1 ±1.7	ND	8.8 ±0.7	ND	25.9 ±2.2
	60	50	16.9 ±1.1	ND	10.2 ±0.8	ND	33.7 ±1.4
	23	46	11.1 ±0.9	ND	9.1 ±0.5	5.5 ±0.3	23.8 ±0.7
	49	43	19.2 ±1.2	ND	11.9 ±0.5	ND	23.4 ±0.8
	52	46	16.1 ±1.1	ND	16.3 ±1.5	ND	38.9 ±3.1
	31	50.5	ND	ND	18.1 ±1.5	5.1 ±0.4	48.2 ±0.9
	22.5	45	117.5 ±7.5	311.1 ±9.8	35.7 ±3.1	5.9 ±0.4	77.8 ±2.4
	28	49.5	10.3 ±0.4	ND	11.1 ±0.7	ND	25.1 ±1.3
Blue veined cheese	52	50	6.4 ±0.1	10.2 ±0.5	72.2 ±4.2	15.4 ±1.2	132.5 ±8.6
	50	52	11.9 ±0.9	ND	127.8 ±4.9	8.3 ±0.6	124.4 ±2.2
	31	53	23.0 ±1.7	ND	87.9 ±2.3	8.4 ±0.2	171.9 ±4.5
	48	48	11.0 ±0.6	ND	40.6 ±2.3	21.1 ±1.9	116.9 ±7.1
	50	52	5.5 ±0.2	ND	220.5 ±11.7	10.5 ±0.8	199.8 ±13.2
	50	53	17.9 ±0.9	ND	66.1 ±5.1	13.8 ±0.5	168.1 ±11.2

* Tryptamine, Phenylethylamine, Histamine ND, fdm – fat in dry matter

veined cheese with fdm 46% w/w. Numbers of lactic acid bacteria were approximately 10^5-10^8 $CFU.g^{-1}$ (lactic acid cocci) and 10^6-10^8 $CFU.g^{-1}$ (*Lactobacillus* spp.). The amount of yeast and mould ranged from 10^5-10^8 $CFU.g^{-1}$.

The total amount of microorganisms in blue veined cheese was higher than the amount in white veined cheese and it was 10^6-10^8 $CFU.g^{-1}$. Presence of enterobacteria was detected only in two samples of blue veined cheese (dry matter 52% w/w, fdm 50% w/w and dry matter 31% w/w, fdm 53% w/w). In the blue veined cheese (dry matter 52% w/w, fdm 50% w/w), the number of enterobacteria was $2,9.10^3$ $CFU.g^{-1}$ and $2,2.10^5$ $CFU.g^{-1}$ in the sample of blue veined cheese (dry matter 48% w/w, fdm 48% w/w). Enterococci were not determined in the blue veined cheese (52% w/w, fdm 50% w/w) while in other cheeses, the amount of enterococci was in order of 10^5-10^7 $CFU.g^{-1}$. The numbers of lactic acid bacteria (both cocci and lactobacilli) were 10^5-10^8 $CFU.g^{-1}$. The amount of yeast and mould in the tested cheese samples ranged from 10^6-10^8 $CFU.g^{-1}$ with the exception of blue veined cheese (dry matter 50% w/w, fdm 53% w/w) where growth of yeast and mould was rather low. Generally, the total numbers of microorganisms in samples of white veined cheese were in lower orders than in samples of blue veined cheese.

Biogenic amines in blue cheese

Tyramine, spermine, putrescine, cadaverine and spermidine are biogenic amines determined in group of blue cheeses. The total amount of biogenic amines in the group was in range 40-600 $mg.kg^{-1}$ with higher amount of BA being determined in blue veined cheese (200-500 $mg.kg^{-1}$). **Komprda et al., (2009)** analyzed blue veined cheese in his study and the same interval for the total amount of BA was presented. In samples of white veined cheese, the total amount of BA reached moderate values <100 $mg.kg^{-1}$, only two samples of this group exceeded this value. **Mayer et al., (2010)** analyzed five samples of the group and the total volume of BA was determined to be >100 $mg.kg^{-1}$ in all tested samples. In our study putrescine was found in 14 samples while, on the other hand, cadaverine was detected only in two samples in amount 10 $mg.kg^{-1}$ and 300 $mg.kg^{-1}$. The total amount of BA in white veined cheese (49.5% fdm) reached value 46.4 $mg.kg^{-1}$. Among all the blue veined cheese, the highest value was reached by the cheese (52% fdm) whose value of BA amount was 436.3 $mg.kg^{-1}$. The concentration of tryptamine and phenylethylamine was low or not detected at all in these cheeses (Table 2.).

Four biogenic amines (tyramine, spermine, spermidine and putrescine) were detected in all samples of the blue veined cheese group. Putrescine and spermidine reached values <100 $mg.kg^{-1}$. Putrescine was determined in all samples of white veined cheese with the exception of one cheese (50.5% fdm). In sample of cheese (45% fdm), the value of putrescine concentration was 117.5 ±7.5 $mg.kg^{-1}$. In sample of white veined cheese (50% fdm), the concentration was 10.17 ±0.5 $mg.kg^{-1}$. On the other hand, tyramine and spermine exceeded this value. The concentration of tyramine in white veined cheese (52%

fdm) was determined to be 220.5 ±11.7 mg.kg^{-1}. The values of spermine exceeding 100 mg.kg^{-1} were found in all samples of this cheese group.

Three biogenic amines-tryptamine, phenylethylamine and histamine-were not detected in any of 15 tested samples. The cadaverine value was 311.13 ±9.6 mg.kg^{-1} in sample of white veined cheese (45% fdm) which was the highest value. Such amount of biogenic amine can be considered toxic. Moreover, cadaverine can increase unwanted toxic effects of the other biogenic amines (histamine and tyramine) or it can inhibit detoxification system, respectively **(Shalaby, 1996)**.

White veined cheeses and blue veined cheeses each contain very different amount of biogenic amines. This amount is rather variable even within one type of cheese or it can be different in various layers of the cheese **(Fernandes, 2008; Buňková et al., 2010)**. The normal amounts of biogenic amines in food and drinks (approximately <100 mg.kg^{-1}) do not pose any significant threat to a healthy human because they are metabolized by detoxification activity of microorganisms in the human intestinal tract **(Halász et al., 1994; Shalaby, 1996)**. **Shalaby (1996)** suggested that the sum of histamine + tyramine + putrescine + cadaverine amounts in cheese should not go beyond 900 mg.kg^{-1}. If the sum of concentration of tested biogenic amines reaches over 200 mg.kg^{-1}, it can be considered as a toxicologically relevant amount **(Halász et al., 1994)**.

SUMMARY

The amount of enterobacteria in fresh cheese exceeded 10^5 CFU.g^{-1}. In smear-ripened cheese flavourless (Romadur type), the amount was >10^3 CFU.g^{-1} and 10^4-10^5 CFU.g^{-1} in smear-ripened cheese with flavour. Enterococci are usually present in various environments. They are very frequent in cheese and they play important role during ripening when they create typical cheese aroma. Formerly, enterococci presence was considered as a sign of poor hygiene. However, it was found out that many enterococci strains are able to produce various antibacterial proteins against pathogenic microorganisms, for example *Listeria monocytogenes*. As non-starter microorganisms, enterococci can also contribute to the development of taste and aroma of the cheese **(Fox et al., 2004; Buňková et al., 2012)**. Number of enterococci was in range 10^4-10^5 CFU.g^{-1} in white veined cheese and 10^5-10^7 CFU.g^{-1} in blue veined cheese. The values of lactic cocci were 10^6-10^8 CFU.g^{-1} in both analyzed cheese groups. Yeast and mould were detected in both groups in amount 10^5-10^8 CFU.g^{-1}. Enterobacteria in food show lack of hygiene during the food production. The most important bacteria in foods are *Escherichia*, *Shigella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Proteus* and *Yersinia*. The presence of enterobacteria was found in 12 samples out of 30 tested cheese types (enterobacteria was not detected in cold-ripened cheese).

The ability to form biogenic amines was described for certain microorganisms, especially *Enterobacteriaceae*, *Pseudomonas* spp. **(Buňková et al., 2010)** and for lactic acid bacteria **(Buňková et al., 2009; Lorencová et al., 2012)**. However, biogenic amines exist more in cheese due to secondary contamination by microorganisms **(Pleva et al., 2012)**. Biogenic amines were studied in two groups: blue cheeses (white veined and blue veined) and smear-ripened cheeses. These cheeses are part of group of soft ripened cheeses. Both groups are considered to contain biogenic amines because the number of microorganisms, as precursors of biogenic amines and free amino acids, is rising during ripening. It is very difficult to determine biogenic amines and polyamines in food. Their amount depends on various external factors. For example, putrescine and cadaverine increase effects of histamine and tyramine. The toxic dose for human organism is not defined exactly and scientist state wide range of possible toxic dose 200-800 mg.kg^{-1} **(Halász et al., 1994; Silla Santos, 1996)**. Some of the analyzed cheeses could pose health risks for a consumer because this amount was exceeded.

These substances can be natural part of materials, for example spermine and spermidine are growth factors and that is why they are present in animal tissue and they can pass into final products **(Kalač and Křížek, 2005)**. Further, biogenic amines can be present starter and non-starter microorganisms. The origin of detected putrescine could be milk used in production because putrescine is natural component of raw milk **(Santos et al., 2003)** or it can be created by decarboxylation of L-arginine and L-ornithine **(Agostinelli et al., 2010; Igarashi and Kashiwagi, 2010; Fuell et al., 2010; Larqué et al., 2007)**. A lot of authors published that lactic acid bacteria (LAB) is one of biogenic amine producers **(Buňková et al., 2009; Ladero et al., 2012; Linares et al., 2011)**. Pasteurized milk was used in production of the cheeses and there may be thermotolerant microorganisms (for example *Enterococcus* genus) in milk modified that way. This group of microorganisms can produce biogenic amines and that is why non-starter microorganisms can be origin of biogenic amines **(Novella-Rodriguez et al., 2004, 2002; Ladero et al., 2010, 2011)**. The presence of histamine and cadaverine can serve as indicator of hygiene during production **(Stadnik and Dolatowski, 2010; Halász et al., 1994)**. Further, biogenic amines can act as contaminants **(Coton et al., 2012)**.

The decrease of biogenic amine amount can be caused by spices and spice mixes because some of them have inhibitory effects on microorganisms and so they can reduce the synthesis of biogenic amines **(Komprda et al., 2004, 2009; Naila, 2010)**.

CONCLUSION

The results of this study show that biogenic amines and polyamines are common in cheese and they can pose a significant health danger for consumers in some cases. They are not monitored by any legislative control authority as they are secondary metabolites even though they are health risks potentially. Biogenic amines were observed in two groups of blue cheeses (white veined cheese and blue veined cheese) and smear-ripened cheeses. Those cheeses belong to group of soft ripened cheese. In both groups, there is a possibility of presence of biogenic amines because the number of microorganisms increases during ripening with the microorganisms posing as precursors for biogenic amines and free amino acids.

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