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Monitoring of biogenic amines in cheeses manufactured at small-scale farms and in fermented dairy products in the Czech Republic



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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 112 samples of dairy products purchased in the Czech Republic, namely in 55 cheeses made in small-scale farms and in 57 fermented dairy products. The products were tested at the end of their shelf-life period. Neither tryptamine nor phenylethylamine was detected in the monitored samples; histamine was found only in four cheese samples containing up to 25 mg/kg. The contents of spermine and spermidine were low and did not exceed the values of 35 mg/kg. Significant amounts of tyramine, putrescine, and cadaverine occurred especially in cheeses produced from ewe's milk or in long-term ripened cheeses. In about 10% of the tested cheeses, the total concentration of all the monitored biogenic amines and polyamines exceeded the level of 200 mg/kg, which can be considered toxicologically significant. In fermented dairy products, the tested biogenic amines occurred in relatively low amounts (generally up to 30 mg/kg) that are regarded safe for the consumer's health.

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1. Introduction

Biogenic amines (BA) are low-molecular nitrogen containing substances known for their characteristic physiological effects. Some BA act as hormones, but many BA are regarded as neurotransmitters or precursors for the synthesis of hormones, alkaloids or other metabolites (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Shalaby, 1996; Silla Santos, 1996).

Most commonly, biogenic amines are formed by decarboxylation of amino acids or by amination or transamination of aldehydes and ketones. Microorganisms demonstrating decarboxylase activity can get into foods spontaneously or they might be contained in starter cultures added intentionally to food (Ladero et al., 2012; Silla Santos, 1996).

Common levels of BA in foodstuffs and beverages (<100 mg/kg), do not represent a serious risk for healthy consumers, because they are metabolised by detoxication enzymes in human intestines. Higher BA amounts can induce undesirable psychoactive and vasoactive effects. Histamine, tyramine, and phenylethylamine might bring about the above adverse effects directly. Putrescine and cadaverine do not show direct toxic effects; however, they can intensify toxic effects of other amines (Halász et al., 1994; Silla

Santos, 1996). If the intestinal detoxication system collapses, because of excessive amount of amines consumed in food or due to its weakening by inhibitors of detoxication enzymes, BA can get into the blood circulation system and may potentially precipitate many undesirable effects (Spano et al., 2010).

Most of the products fermented by lactic acid bacteria (LAB), contain traces of histamine, tyramine, putrescine, and cadaverine. Regarding the occurrence of BA, cheeses are the most important and the most commonly monitored dairy products. The amounts of BA in milk, yoghurts, curd cheese and unripened cheeses can be expected to range between milligrammes and tens of milligrammes per kg (Linares, Martín, Ladero, Alvarez, & Fernández, 2011; Spano et al., 2010).

Recently, studies where products from small-scale farms in south European countries like Italy, Portugal and Greece were characterised, have been published, however, no results of an investigation on relevant products manufactured in central Europe have been reported. Recently, in the Czech Republic, farmer markets have been gaining in popularity. Many Czechs have chosen the healthy life-style inspired by nature, which directs them to the consumption of food produced in small-scale farms. A complex study on the occurrence of BA in products manufactured in the above farms, has not been published yet.

The aim of this study, was to monitor contents of eight BA and polyamines (PA) (histamine, tyramine, 2-phenylethylamine,

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tryptamine, putrescine, cadaverine, spermidine, and spermine) in cheese samples produced in small-scale farms and in selected fermented dairy products at the end of their shelf-life period. The above products were purchased in common Czech retail and/or, in farm markets (including purchase directly in farms).

2. Materials and methods

2.1. Cheese samples and fermented dairy products

Altogether, 55 cheeses manufactured in small-scale farms in the central Europe region (the Czech Republic and the Slovak Republic) were purchased in 2012 (April to July). All of the goods were regional products purchased in farmer markets, in retail network shops or directly in some farms. The purchased cheeses were assorted into the following categories: (i) bryndza cheeses and cheeses made from raw ewe's milk; (ii) cheeses and curd cheeses manufactured from pasteurised ewe's milk; (iii) cheeses produced from goat's milk (all except one, were manufactured from pasteurised milk); (iv) fresh cheeses and curd cheeses produced from

pasteurised cow's milk; and (v) ripened cheeses made from pasteurised cow's milk (Table 1).

Besides the above cheeses, 55 fermented dairy products purchased in common Czech and Slovak retail network shops and two fermented dairy products manufactured at small-scale farms based in east Moravia (Czech Republic) were analysed. The following fermented products were monitored: (i) 29 yoghurts (one cream yoghurt was produced at a farm); (ii) 14 fermented creams; (iii) 4 acidified milks – milks fermented with an acidophilic culture; (iv) 4 kefir milks; (v) 2 fermented buttermilks; and (vi) 4 fermented milks – milks fermented with mesophilic cultures (one made at a farm) (Table 2). None of the monitored fermented products contained flavouring.

The cheese samples and fermented milk products were kept at cold store temperatures of 6 ± 2 °C. They were analysed at the end of their shelf-life period.

2.2. Determination of biogenic amines and polyamine contents

If necessary, all the collected samples were homogenised, and subsequently lyophilised (Christ Alpha 1–4 device). The amines

 Table 1

 Content of biogenic amines in mg/kg in tested cheeses produced at small-scale farms.

Cheese	Number of samples	Biogenic amines (mg/kg) ^a					
		Total BA	Tyramine	Histamine	Putrescine	Cadaverine	Spermine
Raw ewe's milk cheeses							
Bryndza	4	73.2-222.2	4/34.6-107.4	1/24.2 ± 1.1	2/22.1-60.9	3/16.5-42.6	$1/9.7 \pm 0.8$
Smoked cheese	3	25.1-177.1	3/8.9-38.3	ND^b	3/16.2-99.9	2/62.6-80.7	ND
Fresh cheese	2	NDb-40.3	ND	ND	1/20.7 ± 1.3	1/19.6 ± 1.4	ND
Pasteurised ewe's milk cheeses							
Unripened (fresh) cheese	3	ND-140.3	2/10.2-11.1	ND	2/55.3-118.2	2/11.4-35.8	ND
Pasta filata type cheese	2	ND-13.2	ND	ND	ND	ND	1/13.2 ± 1.1
Brined cheese	2	37.2-529.8	2/23.1-174.6	ND	1/229.5 ± 20.0	1/125.6 ± 3.0	1/14.0 ± 1.1
Flavoured cheese	2	ND-223.5	1/114.7 ± 8.0	ND	1/108.8 ± 7.0	ND	ND
Goat's milk cheeses							
Ripened cheese (raw milk without heat treatment)	1	356.1	1/207.1 ± 7.5	ND	ND	1/149.0 ± 7.3	ND
Unripened (fresh) cheese (unflavoured)	3	ND-32.7	1/11.3 ± 1.0	ND	ND	ND	ND
Unripened (fresh) cheese (flavoured)	6	ND-10.7	1/10.7 ± 0.5	ND	ND	ND	ND
Pasta filata type cheese (plant)	3	ND-89.9	1/8.5 ± 0.6	ND	1/41.1 ± 3.3	1/40.3 ± 2.5	ND
Fresh cow's milk cheeses							
Unripened (fresh) cheese (unflavoured)	6	ND-161.9	2/8.3-15.2	ND	1/111.4 ± 8.6	3/8.9 ± 21.7	4/11.4-17.9
Unripened (fresh) cheese (flavoured)	4	19.4-45.1	3/7.2-22.7	ND	ND	2/7.0-22.4	3/11.8–15.5
Other cow's milk cheeses							
Ripened cheese	3	137.9-173.4	2/63.8-101.4	ND	2/48.8-70.1	3/11.8-72.6	3/11.5-97.9
Pasta filata type cheese (unflavoured)	5	ND-49.9	1/25.8 ± 1.5	1/19.3 ± 0.6	1/37.3 ± 1.1	ND	2/7.1–12.7
Pasta filata type cheese (flavoured)	3	12.0-25.6	ND	ND	2/12.2-14.7	ND	3/10.9-12.0
Pasta filata type cheese (unflavoured, smoked)	3	9.2-39.3	ND	2/18.3-18.8	ND	1/10.7 ± 0.9	2/9.2-9.8

^a Individual biogenic amines – number of BA positive samples/range of detected BA amount.

Table 2Content of biogenic amines in mg/kg in fermented dairy products.

Product	Number of samples	Biogenic amines (mg/kg) ^a					
		Total BA	Tyramine	Putrescine	Cadaverine		
Yoghurt (fat content 3–10%)	17	0.5-29.4	17/0.3-6.3	8/1.9-25.1	ND ^b		
Yoghurt (fat content 10-11%)	4	ND ^b -26.6	3/0.5-2.7	2/3.2-26.1	ND		
Yoghurt (fat content 2-3%)	3	1.1-13.3	3/1.1-1.7	$1/1.4 \pm 0.2$	$1/4.3 \pm 0.3$		
Yoghurt (fat content $\approx 0.1\%$)	5	1.5-10.5	4/0.3-2.0	3/5.0-10.5	ND		
Fermented cream	14	5.5-15.4	12/5.5-15.4	2/8.6-8.8	ND		
Acidified milk	4	2.9-7.5	3/2.6-7.5	$1/4.1 \pm 0.3$	ND		
Fermented milk	4	3.2-6.2	3/3.2-6.2	ND	ND		
Fermented buttermilk	2	4.6-5.0	2/4.6-5.0	ND	ND		
Kefir milk	4	3.4-15.3	3/0.8-4.0	3/3.4-14.3	ND		

^a Individual biogenic amines – number of BA positive samples/range of detected BA amount.

^b ND – biogenic amines not detected.

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