Contents of biogenic amines and polyamines in mould-fermented sausages

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Summary

The aim of this work was to determine the contents of biogenic amines (BA) and polyamines (PA) in dry fermented sausages of a small diameter (< 40 mm) with surface moulds, and to compare it with analogical data for smoked dry fermented sausages. The pH value, BA and PA contents were examined in samples taken on days 0 and 29 (fuet) and days 0 and 28 (Hungarian sausage), and were compared to the parameters obtained for smoked sausages (Nitran). The analysis of the content of BA in mould-fermented sausages with a small casing diameter did not demonstrate different contents of these substances in comparison with traditional smoked products available on the market. The effect of low pH values in stimulating the formation of BA was not proven. The values of BA and PA found in all the samples analysed did not exceed 200 mg·kg^{-1}. The BA contained in the largest quantities in fuet sausages were tyramine and putrescine, while larger quantities of tyramine and histamine were found in Hungarian-type sausages.

Keywords

fuet; Hungarian-type sausage; pH value; tyramine; putrescine; cadaverine

The popularity of dry fermented sausages has been on the increase in the countries of Central Europe in the last two decades. While in 1988 around 9 000 tons of these products were made in the whole of the former Czechoslovakia [1], production in the Czech Republic in 2009 was estimated at more than 20 000 t annually [2]. The great majority of these products belongs to the group of smoked sausages. In the mid 1990s, some producers introduced products with surface moulds. These are mostly sausages from 60 mm to 65 mm in diameter, filled in artificial collagen casings. They are generally products of the Hungarian type. The market in meat products also offers mould-fermented sausages with a casing of a smaller diameter (35–38 mm) as an alternative to traditional products. Products of a diameter of less than 40 mm became more popular in recent years among both consumers and producers. Their ripening period is shorter thanks to the favourable surface–weight ratio, the piece price of whole products on the market is lower, and the sausages are characterized by pronounced sensory properties, particularly with regard to texture.

The strains of cultural moulds that intentionally cover the surface of unsmoked sausages give these products specific properties caused by the metabolic activity of the moulds, whose hydrolytic enzymes break down fats, proteins and lactic acid in the sausages [3], as a result of which products with surface moulds generally attain a higher pH than traditional smoked sausages. The pH value is a barrier to the growth of undesirable bacteria during the course of ripening of dry fermented sausages. In spite of the higher pH of mould-fermented sausages (> 5.2) these products are stable and safe, as they have a low a_w value due to the long ripening phase.

The factors that may have a negative influence on the nutritional value of dry fermented sausages include biogenic amines and polyamines [4]. A number of factors that may affect the for-

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mation of biogenic amines during the course of sausage fermentation, such as pH, the value of a_W, the NaCl content, the starter culture used, the period of maturing and the temperature during maturing, have been studied [5–8]. Although the issue of biogenic amines in fermented sausages has been extensively studied and has been the subject of review [7, 9, 10], data on mould-fermented sausages of small diameter are available only for Spanish [11, 12] or Italian [13] products. The formation of biogenic amines in small-diameter sausages with surface mould of Czech or Hungarian production has not been studied to date.

The aim of this work was to determine the contents of biogenic amines in dry fermented sausages a small diameter (< 40 mm) with surface moulds and to compare it with analogical data from smoked dry fermented sausages.

MATERIAL AND METHODS

Sausage preparation

Fuet

Fuet sausage was prepared in plant A in the Czech Republic. The recipe for 100 kg of the final product included 80 kg of chilled pork shoulder, 20 kg of frozen pork trimmings 80/20 (20% fat content), and 40 kg of pork back fat. The batter was supplied with 2.5% nitrite curing salt, 0.3% black ground pepper and 0.05% sodium isoascorbate. Two batches differing in additive starter cultures and glucose content were made. Batch 1 contained 0.1% glucose and CS300 (starter culture containing a *Staphylococcus carnosus* strain) from the company Chr. Hansen (Chr. Hansen, Nienburg, Germany). Batch 2 contained 0.3% glucose and BFL F04 (starter culture with strains *Staphylococcus carnosus* and *Lactobacillus sakei;* Chr. Hansen). 3 samples from each batch were analysed. The batter was mixed to form 3–5 mm granules and filled in collagen casings of 35 mm in diameter. The sausages were 250 mm long. Immediately after filling, the products were immersed in a solution of mould spores containing a culture of *Penicillium nalgiovense* (Mould 700; Chr. Hansen). The sausages were dispatched on the 29th day after filling.

The fermentation and ripening process took place under the external conditions that are specified in Tab. 1.

Hungarian-type sausage

The preparation of Hungarian-type sausage was performed in plant B in Hungary. The traditional Hungarian sausages (batches 1–4; 3 samples from each batch were analysed) were made of lean sow meat (60 kg) and pork belly (40 kg), with nitrite salt (2.5 kg), spice, glucose and a starter culture (BFL F04). The batter was filled in collagen casings (38 mm in diameter). The fermentation and ripening processes were conducted under external conditions same as those for fuet sausage (Tab. 1). The ripening period was 28 days.

Nitran

Samples A1–A4 of Nitran sausage were taken from a Slovakian producer of meat products (producer C) in order to compare the content of biogenic amines (BA) and polyamines (PA) in mould-fermented sausages and smoked sausages. Nitran sausage is made from a mix of pork and beef meat with added pork fat. A starter culture (Bactoferm, F-SC-111, Chr. Hansen), glucose and a mixture of spices are added to the batter. The batter is filled in collagen casings of a diameter of 55 mm. The period of maturing does not generally exceed 28 days. The product is smoked with cold smoke in the first week. In our experiment, 42 kg of pork back fat and 98 kg of lean meat consisting of 42 kg of sow leg (A1 and A2) or shoulder of pork (A3 and A4), 51 kg of pork trimmings (fat content of 25%) and lean beef meat (5 kg), were used to make 140 kg of batter in batches A1–A4 (3 samples from each batch were analysed). Fermentation and ripening took place in air-conditioned chambers under conditions (temperature, relative air humidity) as for the fuet sausages (Tab. 1).

pH values analyses

pH values were determined in samples of fuet sausage taken on days 0 and 29. Samples of Hun-

Tab. 1. Microclimatic conditions during fuet, Hungarian-type and Nitran sausages ripening.

Day of ripening	Temperature in chamber [°C]	Relative air humidity in chamber [%]
1	24	94
2	22	93
3–4	22	92
5	20	92
6	20	90
7	18	90
8	17	88
9–11	16	85
12–19	16	80
20–24	16	78
25–28/29	16	75

garian sausage and Nitran sausage were taken on day 28 (Nitran sausage also on day 0). pH values were measured with a Double Pore needle probe (Hamilton, Bonaduz, Switzerland) on a 340i WTW pH-meter (WTW, Weilheim, Germany). Each sample of sausage was measured three times.

Determination of biogenic amines and polyamines

BA content was examined in the samples taken on days 0 and 29 (fuet) and days 0 and 28 (Hungarian sausage, Nitran sausage). Biogenic amines (agmatine, tryptamine, 2-phenylethylamine, cadaverine, histamine, tyramine) and polyamines (putrescine, spermidine and spermine) were determined by the method of PAULSEN et al. [14]. Biogenic amines and polyamines were extracted from the food matrix with 10% (w/v) trichloracetic acid and subsequently detected as dansyl derivatives. Quantitative analysis was performed by the RP-HPLC method with gradient elution and fluorescence detection; histamine was determined using a photodiode array detector (PDA).

The samples were analysed in an Alliance 2695 chromatograph (Waters, Milford, Illinois, USA) with PDA 2996 detectors and fluorescence detector 2495, using a Zorbax Eclipse XDB C18 column (Agilent, Santa Clara, California, USA), 150 × 4.6 mm, 5 μm, with a Zorbax 30 × 4.6 mm, 5-μm pre-column. Separation took place in the gradient elution mode, mobile phase A being represented by a mixture of 0.1 mol·l^{-1} acetic acid and acetonitrile (90 : 10), mobile phase B by a mixture of 0.1 mol·l^{-1} acetic acid, acetonitrile and methanol (10 : 45 : 45). The flow rate was 1 ml.min^{-1} with feeding of 10 μl. Detection was performed at $\lambda_{ex}/\lambda_{em}$ = 330/500 nm, detection within the UV region at 254 nm. Each sample was analysed in at least two tests, with a blind sample included in each series. Separated biogenic amines and polyamines were identified by means of a comparison of their retention times with standard retention times. These biogenic amines were used as standards: agmatine, tryptamine, 2-phenylethylamine, cadaverine, histamine and tyramine. These polyamines were used as standards: putrescine, spermidine and spermine. Standard solutions were prepared by dissolving 10–50 mg (calculated on the pure substance basis) in 0.1 mol·l^{-1} HCl, the final volume being 25 ml. Solutions were freshly made every 14 days and stored at 4 °C in a dark place.

The measurements were evaluated with the help of Empower Fluorescence software (Waters). Limit of quantification (*LOQ*) of all BA and PA was 0.1 mg·kg^{-1}.

Statistical evaluation

Statistical data analyses were conducted using the statistical programme Statistica 7 CZ (StatSoft, Prague, Czech Republic). Quality parameters were tested for normal distribution in each group of sausages. Kruskal-Wallis test was used, followed by multiple comparison to find out the pairs of groups with different values. A significance level of 0.05 was used.

RESULTS

The pH values for fuet sausages, Hungarian-type sausages and Slovakian Nitran sausages are given in Tab. 2–4. The highest pH values were measured in fuet sausages (6.74 and 6.89), while the lowest values were recorded in smoked Nitran products (4.70–4.82).

The results for the content of BA and PA in samples of sausage are given in Tab. 2–4. The content of BA and PA was increased from the initial 20–25 mg·kg^{-1} in the raw material mixture to 38–183 mg·kg^{-1} in the final products after 28 or 29 days of ripening.

Histamine, tyramine, spermine and putrescine were predominant in the batter prepared from a mixture of meat and additives, accounting for a proportion of 86–97.8% of the total contents of BA and PA. The proportion accounted for by the four above BA and PA was higher (97.8% and 97.1%) in the batter for fuet sausages.

The proportion of BA and PA changed during the ripening. The individual groups of products differed not only in terms of the final contents of these metabolites, but also in respect of their proportions. In fuet sausages, the highest content was shown by tyramine, followed by putrescine, histamine and spermine or cadaverine (Tab. 2). In Hungarian-type sausage, the highest proportion was also accounted for by tyramine, the second highest by histamine (or 2-phenylethylamine), followed by 2-phenylethylamine (or histamine) and tryptamine (Tab. 3). In Nitran smoked sausages, the highest values were shown by histamine, cadaverine and putrescine/tyramine, though the content of agmatine was also high (Tab. 4). In the final products, therefore, the highest contents were of tyramine, histamine, putrescine and cadaverine. They accounted for a proportion of approximately 83–85% of the total content in fuet sausages, 62–83% in Hungarian products, and 73–81% in Nitran sausages.

The level of BA and PA increased most during the ripening in sample A1 (Nitran) – almost 9 times. A strong increase was also seen in both

Tab. 2. pH and contents of biogenic amines and polyamines in fuet sausage.

Batch	Day	pH	TRY	AGM	2-P	PUT	CAD	HIS	TYR	SPD	SPN	Total amines
			[mg·kg^{-1}]									
FS1	0	5.95 ± 0.02^a	< *LOQ*^{a}	< *LOQ*^{a}	0.40 ± 0.01^a	2.24 ± 0.55^a	< *LOQ*^{a}	10.64 ± 0.16^a	4.49 ± 0.23^a	0.10 ± 0.00^a	2.25 ± 0.21^a	20.12
	29	6.74 ± 0.01^b	4.14 ± 0.20^b	2.12 ± 0.16^b	1.82 ± 0.09^b	40.88 ± 2.23^b	2.86 ± 0.14^b	13.75 ± 0.43^b	49.13 ± 1.24^b	3.17 ± 0.16^b	7.28 ± 0.14^b	125.15
FS2	0	6.02 ± 0.01^a	< *LOQ*^{a}	< *LOQ*^{a}	0.41 ± 0.00^a	2.76 ± 0.01^a	< *LOQ*^{a}	10.82 ± 0.01^a	4.88 ± 0.00^a	0.27 ± 0.09^a	4.01 ± 0.66^a	23.15
	29	6.89 ± 0.01^b	5.78 ± 0.24^b	7.35 ± 0.80^b	4.78 ± 0.48^b	49.77 ± 4.18^b	14.36 ± 1.03^b	14.46 ± 1.14^b	60.77 ± 2.00^b	3.05 ± 0.50^b	7.73 ± 1.46^b	168.05

Values are expressed as an average ± standard deviation.
FS1 – fuet sausage batch 1, FS2 – fuet sausage batch 2, *LOQ* – limit of quantification, TRY – tryptamine, AGM – agmatine, 2-P –2-phenylethylamine, PUT – putrescine, CAD – cadaverine, HIS – histamine, TYR – tyramine, SPD – spermidine, SPN – spermine.
a, b – statistical significant differences ($P \leq 0.05$) in one product between days (0/29 day) (in column).

Tab. 3. pH and contents of biogenic amines and polyamines in Hungarian-type sausage.

Batch	Day	pH	TRY	AGM	2-P	PUT	CAD	HIS	TYR	SPD	SPN	Total amines
			[mg·kg^{-1}]									
HTS1	0	–	–	–	–	–	–	–	–	–	–	–
	28	5.78 ± 0.02	2.41 ± 0.02	< *LOQ*	21.23 ± 1.23	< *LOQ*	< *LOQ*	10.75 ± 0.26	33.95 ± 5.19	0.22 ± 0.15	3.06 ± 1.00	71.62
HTS2	0	–	2.22 ± 0.01^a	0.17 ± 0.00^a	0.39 ± 0.01^a	3.30 ± 3.08^a	< *LOQ*^{a}	10.52 ± 0.04^a	3.86 ± 0.05^a	0.19 ± 0.13^a	3.91 ± 1.14^a	24.56
	28	5.18 ± 0.02	2.25 ± 0.01^a	0.12 ± 0.03^a	3.72 ± 0.04^b	1.14 ± 0.09^a	< *LOQ*^{a}	10.50 ± 0.39^a	18.50 ± 1.00^b	0.10 ± 0.05^a	2.06 ± 0.31^a	38.39
HTS3	0	–	2.22 ± 0.01^a	< *LOQ*^{a}	0.39 ± 0.01^a	0.20 ± 0.01^a	< *LOQ*^{a}	10.38 ± 0.05^a	3.73 ± 0.02^a	0.13 ± 0.06^a	3.16 ± 0.22^a	20.21
	28	5.49 ± 0.01	2.27 ± 0.00^b	0.10 ± 0.02^a	3.05 ± 0.05^b	0.94 ± 0.05^b	< *LOQ*^{a}	10.58 ± 0.39^a	30.53 ± 0.61^b	0.16 ± 0.01^a	3.13 ± 0.01^a	50.76
HTS4	0	–	2.21 ± 0.00^a	< *LOQ*^{a}	0.41 ± 0.00^a	0.64 ± 0.06^a	< *LOQ*^{a}	10.58 ± 0.20^a	3.79 ± 0.16^a	0.22 ± 0.02^a	2.39 ± 1.29^a	20.24
	28	5.79 ± 0.03	2.27 ± 0.00^b	< *LOQ*^{a}	4.85 ± 0.48^b	< *LOQ*^{a}	0.10 ± 0.01^a	10.36 ± 0.03^a	24.31 ± 2.51^b	< *LOQ*^{a}	1.65 ± 0.29^a	43.54

Values are expressed as an average ± standard deviation.
HTS1 – Hungarian-type sausages batch 1, HTS2 – Hungarian-type sausages batch 2, HTS3 – Hungarian-type sausages batch 3, HTS4 – Hungarian-type sausages batch 4, *LOQ* – limit of quantification, TRY – tryptamine, AGM – agmatine, 2-P –2-phenylethylamine, PUT – putrescine, CAD – cadaverine, HIS – histamine, TYR – tyramine, SPD – spermidine, SPN – spermine.
a, b – statistical significant differences ($P \leq 0.05$) in one product between days (0/28 day) (in column).
a, a – no statistical significant differences ($P > 0.05$) in one product between days (0/28 day) (in column).

Tab. 4. pH and contents of biogenic amines and polyamines in Nitran sausage.

Batch	Day	pH	TRY	AGM	2-P	PUT	CAD	HIS	TYR	SPD	SPN	Total amines
			[mg·kg^{-1}]									
NSA1	0	5.79±0.03^{a}	2.23±0.01^{a}	0.18±0.14^{a}	0.46±0.03^{a}	0.16±0.22^{a}	$< LOQ^{a}$	10.82±0.08^{a}	3.87±0.12^{a}	0.12±0.05^{a}	3.63±0.14^{a}	21.47
	28	4.7 ±0.02^{b}	2.42±0.04^{b}	36.18±1.35^{b}	1.28±0.02^{b}	29.63±0.71^{b}	35.27±0.32^{b}	51.23±1.64^{b}	23.82±2.83^{b}	0.10±0.01^{a}	3.21±0.04^{a}	183.14
NSA2	0	5.82±0.02^{a}	$< LOQ^{a}$	$< LOQ^{a}$	0.42±0.01^{a}	1.54±1.50^{a}	$< LOQ^{a}$	10.91±0.37^{a}	3.88±0.22^{a}	0.43±0.41^{a}	6.16±0.04^{a}	23.34
	28	4.8 ±0.04^{b}	2.52±0.09^{b}	20.24±1.38^{b}	0.88±0.12^{b}	22.72±4.25^{b}	30.65±4.78^{b}	34.85±2.82^{b}	29.65±14.10^{a}	0.11±0.15^{a}	3.28±0.76^{a}	144.90
NSA3	0	5.83±0.02^{a}	2.23±0.01^{a}	0.10±0.02^{a}	0.47±0.01^{a}	1.48±0.13^{a}	$< LOQ^{a}$	10.77±0.20^{a}	3.78±0.14^{a}	0.16±0.23^{a}	3.90±1.48^{a}	22.89
	28	4.82±0.02^{b}	2.36±0.03^{b}	11.67±1.27^{b}	0.53±0.02^{b}	10.20±5.58^{b}	12.86±0.52^{b}	16.94±1.56^{b}	10.25±0.79^{b}	0.20±0.03^{a}	4.13±0.18^{a}	69.14
NSA4	0	5.79±0.03^{a}	2.31±0.01^{a}	0.59±0.48^{a}	0.42±0.00^{a}	1.48±2.09^{a}	$< LOQ^{a}$	12.51±0.40^{a}	4.07±0.07^{a}	0.18±0.09^{a}	4.09±0.80^{a}	25.65
	28	4.78±0.03^{b}	2.43±0.03^{b}	6.12±1.90^{a}	1.79±1.72^{a}	4.03±0.09^{a}	6.05±0.43^{b}	19.07±0.46^{b}	11.84±1.80^{b}	0.12±0.13^{a}	3.92±0.11^{a}	55.37

Values are expressed as an average ± standard deviation.
NSA1 – Nitran sausages batch A1, NSA2 – Nitran sausages batch A2, NSA3 – Nitran sausages batch A3, NSA4 – Nitran sausages batch A4, *LOQ* – limit of quantification, TRY – tryptamine, AGM – agmatine, 2-P –2-phenylethylamine, PUT – putrescine, CAD – cadaverine, HIS – histamine, TYR – tyramine, SPD – spermidine, SPN – spermine.
a, b – statistical significant differences ($P \leq 0.05$) in one product between days (0/28 day) (in column).
a, a – no statistical significant differences ($P > 0.05$) in one product between days (0/28 day) (in column).

samples of fuet sausages (6 times and 8 times). Products of the Hungarian type, in contrast, had extremely low contents of BA and PA, and these values increased by a factor of just 1.5–2.5 over the initial values during 4 weeks.

A content of 100.00 mg·kg^{-1} was exceeded in four cases – both samples of fuet sausage (125.15 mg·kg^{-1} and 168.05 mg·kg^{-1}) and samples A1 and A2 (Nitran; 183.05 mg·kg^{-1} and 144.9 mg·kg^{-1}). The value determined in sample A1 was the highest content among all samples. Statistically significant differences between the individual types of sausage are in Tab. 2–4. Statistically significant differences between the ready products (day 28/29) are given in Tab. 5.

DISCUSSION

Spermidine and spermine, and to a lesser extent putrescine, predominate in meat for the production of dry fermented sausages and, thereby, in the batter for filling in sausage casings [7]. During the storage of meat, the contents of cadaverine and putrescine increase, while the contents of spermidine and spermine decrease [15]. Where a higher content of other BA is also demonstrated, this points to microbial contamination of the input raw materials. In all the groups of samples analysed in this work, the highest content in the sausage batter immediately after filling in sausage casings was shown by histamine, followed by tyramine, spermine and putrescine. Roseiro et al. [4] recorded spermine, tyramine and histamine as accounting for the highest proportion in the batter of Portuguese smoked dry fermented sausages. These authors were unable to demonstrate the presence of other BA.

The highest contents found in final fermented sausages are generally of tyramine and putrescine [16, 17]. A high level of cadaverine and, to a lesser extent, histamine was also shown in certain samples, though the presence of these BA is put into context with the lower quality of meat as a result of proliferation of the contaminating microflora [7, 12].

In the fuet sausages analysed in this study, the BA most heavily represented included tyramine and putrescine, in Hungarian-type sausages the largest quantities found were of tyramine and histamine, while in Nitran smoked products histamine predominated, followed by cadaverine and agmatine, with the biogenic amines putrescine and tyramine being found in smaller quantities. The results presented are

Tab. 5. Statistically significant differences between individual types of sausage with respect to the contents biogenic amines.

	Fuet sausages × Nitran sausages		Fuet sausages × Hungarian sausages		Nitran sausages × Hungarian sausages	
Day	0	28/29	0	28/29	0	28/29
pH	$P \leq 0.05$	$P \leq 0.05$	–	–	–	
TRY				$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$
AGM						$P \leq 0.05$
2-P					$P \leq 0.05$	$P \leq 0.05$
PUT				$P \leq 0.05$		
CAD						$P \leq 0.05$
HIS					$P \leq 0.05$	$P \leq 0.05$
TYR	$P \leq 0.05$	$P \leq 0.05$	$P \leq 0.05$			
SPD		$P \leq 0.05$				
SPM				$P \leq 0.05$		$P \leq 0.05$

TRY – tryptamine, AGM – agmatine, 2-P –2-phenylethylamine, PUT – putrescine, CAD – cadaverine, HIS – histamine, TYR – tyramine, SPD – spermidine, SPN – spermine.

in agreement with the data on the Portuguese dry fermented sausage "Chouriço Grosso de Estremoz e Borba PGI", in which putrescine, cadaverine and tyramine predominated after the third week of maturing [4], similarly as for fuet sausage [18].

It is clear from the results that fuet sausages showed the highest pH values and a high BA content at the end of the ripening process. The pH value is a key factor influencing the decarboxylation activity [7]. Organic acids support the aminogenic activity of microorganisms, as bacteria release BA probably as a means of defence against an acid environment, thereby serving to maintain the intracellular homeostasis [8]. Amino acid decarboxylation is stronger in an acidic environment [19]. Nevertheless, formation of BA depends to a greater extent on proliferation of microorganisms with the appropriate decarboxylation activity than on the growth conditions as such [7]. Certain strains of lactic acid bacteria with the ability of amino acid decarboxylation may be metabolically active in the batter of dry fermented sausages [20]. Komprda et al. [21] identified the species *Lactobacillus plantarum, Lb. brevis*, *Lb. casei/paracasei*, *Enterococcus faecium* and *Enterococcus faecalis* with the ability to produce tyramine or histamine in the batter of dry fermented sausages.

Because lower pH values inhibit the growth of numerous bacteria and represent a barrier to undesirable microbes, and in particular to representatives of the family *Enterobacteriaceae,* acidification of sausages can be used as a tool controlling the course of fermentation and, thereby, the quality of the ready products [22]. In the case of mould-fermented sausages, however, the metabolic activity of the surface moulds increases the pH value [3]. Ansorena et al. [16] discovered a higher content of BA in dry fermented sausages with surface moulds as compared with smoked products from northern Europe. A higher content of ammonia, which increases the pH, occurs in mould-fermented sausages as a result of proteolysis. The surface moulds also metabolize lactic acid, the content of which falls in the batter, which also leads to an increase in pH. The correlation between pH and the content of biogenic amines and polyamines was studied in the work presented here. A positive statistically significant correlation ($r = 0.668$, $P \leq 0.05$) was found between pH and the content of histamine, while in contrast, negative statistically significant values of correlation coefficients were determined for tryptamine ($r = -0.803$, $P \leq 0.05$) and agmatine ($r = -0.778$, $P \leq 0.05$).

On the other hand, proteolysis releases amino acids – precursors of biogenic amines. This might logically suggest that a higher content of amino acids leads to a higher probability of the formation of BA. This is, at a first glance, something of a paradox – the formation of BA in the sausage batter may be stimulated by a lower pH, while on the other hand proteolysis, which increases the pH, releases precursors to BA into the environment.

The given results showed a higher level of BA in fuet mould-fermented sausages. The content of BA was significantly lower in Hungarian-type sausages prepared with identical mould cultures, and did not greatly differ from the quantity detected in the raw material mixture.

In smoked Nitran sausages, whose pH in the final product did not exceed 5.0, the content of BA showed values extremely similar to fuet sausages in two cases (183.05 mg·kg^{-1} and 144.9 mg·kg^{-1}). Samples A3 and A4, in contrast, showed levels of BA and PA similar to those in Hungarian products. The pH evidently does not have such an influence on the formation of BA during the ripening of dry fermented sausages as might be expected. The influence of proteolysis may also be overestimated. BOVER-CID et al. [23] did not find a positive correlation between the value of the proteolysis index or non-protein nitrogen and the formation of BA in fuet sausages. Nitran products differed in terms of the type of ingredients used (A1 and A2 versus A3 and A4), which might have an influence on the final content of BA, even though such a difference was not evident in the raw material mixture prepared.

The values of BA and PA determined in samples of fuet sausages, sausages of the Hungarian type and Nitran products were all relatively low. The level of tyramine ranged from 10.25 mg·kg^{-1} (Nitran) to 60.77 mg·kg^{-1} (fuet). No putrescine at all could be detected in some final products (Hungarian-type sausages), with the highest content being found in fuet sausages (49.77 mg·kg^{-1}). In their review paper, SUZZI and GARDINI [7] state findings of tyramine in dry fermented sausages from various European countries ranging from zero to 742.6 mg·kg^{-1} (fuet). PAULSEN et al. [24] stated that the highest level of tyramine in dry fermented sausages in Austria was 433 mg·kg^{-1}. ANSORENA et al. [16] detected levels of tyramine of more than 100.0 mg·kg^{-1} in sausages with surface mould, and levels of putrescine from zero to 500.7 mg·kg^{-1} (the product Sobrasada). Histamine, which predominated in Nitran sausages (51.23 mg·kg^{-1} and 34.85 mg·kg^{-1} in samples A1 and A2), reached contents of between 10 mg·kg^{-1} and 14.5 mg·kg^{-1} in mould-fermented sausages. The values published in the literature are as much as 314.3 mg·kg^{-1} (chorizo), 357.0 mg·kg^{-1} (fuet) [7] and 400.0 mg·kg^{-1} of dry matter in Austrian sausages [16].

The finding of a relatively high level of agmatine in final products of the Nitran type is interesting. To date, little data are available on the presence of agmatine in meat and meat products. This BA was first mentioned in relation to the proposal of agmatine as an indicator of the quality of seafood [25], and later as an indicator of spoilage of fish and meat [26]. Agmatine is the first BA detected at the start of spoilage of meat and fish, and its content then falls to an undetectable level [27].

We discovered a considerable variability in the agmatine content in fermented meat products at the end of maturing (days 28–29) in our study. The content of agmatine ranged from values below the level of quantification up to a content of 36.2 mg·kg^{-1} in Nitran sausages. This content is comparable with the value of 34.8 mg·kg^{-1} of dry matter shown at the end of maturing for fuet sausages [28]. These authors reported on an intensive agmatine production during maturing for a period of 24 days, with the formation of agmatine being observed as early as during the first week of maturing. Its content increased gradually during the course of the maturing process. At the end of maturing, however, the agmatine content fell slightly from values of 45.2 mg·kg^{-1} of dry matter to the stated 34.8 mg·kg^{-1} of dry matter. The considerable variability in the agmatine content observed in our study was seen not merely during the comparison of various types of fermented sausage (fuet, Hungarian sausage), but also between individual batches from the same producer (Nitran, Hungarian sausage). A similar phenomenon was described by BOVER-CID et al. [17] and HUI [29].

The quality of meat used in production has evidently the greatest influence on the content of BA in final products in the category dry fermented sausages. Microbial contamination represents the potential for a greater occurrence of bacterial enzymes that remain active during the course of the maturing process regardless of the actual number of microorganisms [7] and that may increase the content of BA in products destined for the market. Measures taken to reduce the BA content in dry fermented sausages must, therefore, focus first and foremost on the quality and freshness of meat used in production.

CONCLUSION

The analysis of the contents of biogenic amines and polyamines in mould-fermented sausages with a small casing diameter did not demonstrate a differing content of these substances in comparison with traditional smoked products available on the Slovakian market. The effect of low pH values in stimulating the formation of BA was not proven. The values of BA and PA found in all samples were relatively low. The BA contained in the largest quantities in fuet sausages were tyramine and putrescine, while larger quantities of tyramine and histamine were found in Hungarian-type sausages; histamine predominated in Nitran smoked products, followed by cadaverine and agmatine, with the biogenic amines putrescine and tyramine being found in smaller quantities. The influence of the microbiological quality of meat has evidently a far larger influence on the BA content in the final dry fermented sausages than the technology used in the production process.

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REFERENCES

1. Lücke, F. K. – Brümmer, J. M. – Buckenhüskes, H. – Garrido, F. A. – Rodrigo, M. – Smith, J. E.: Starter culture development. In: Zeuthen, P. – Cheftel, J. C. – Eriksson, C. – Gormley, T. R. – Linko, P. – Paulus, K.: Processing and quality of foods. Vol. 2. Food Biotechnology: Avenues to healthy and nutritious products. London, New York : Elsevier Applied Science, 1990, pp. 2.11–2.36.
2. Kameník, J. – Saláková, A. – Bořilová, G. – Pavlík, Z. – Standarová, E. – Steinhauser, L.: Effect of storage temperature on the quality of dry fermented sausage Poličan. Czech Journal of Food Sciences, *30*, 2012, pp. 293–301.
3. Sunesen, L. O. – Stahnke, L. H.: Mold starter cultures for dry sausages – selection, application and effects. Meat Science, *65*, 2003, pp. 935–948.
4. Roseiro, L. C. – Gomes, A. – Gonçalves, H. – Sol, M. – Cercas, R. – Santos, C.: Effect of processing on proteolysis and biogenic amines formation in a Portuguese traditional dry-fermented ripened sausage "Chouriço Grosso de Estremoz e Borba PGI". Meat Science, *84*, 2010, pp.172–179.
5. Gardini, F. – Martuscelli, M. – Caruso, M. C. – Galgano, F. – Crudele, M. A. – Favati, F. – Guerzoni, M. E. – Suzzi, G.: Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of *Enterococcus faecalis*. International Journal of Food Microbiology, *64*, 2001, pp. 105–117.
6. Gardini, F. – Zaccarelli, A. – Belletti, N. – Faustini, F. – Cavazza, A. – Martuscelli, M. – Mastrocola, D. – Suzzi, G.: Factors influencing biogenic amine production by a strain of *Oenococcus oeni* in a model system. Food Control, *16*, 2005, pp. 609–616.
7. Suzzi, G. – Gardini, F.: Biogenic amines in dry fermented sausages: a review. International Journal of Food Microbiology, *88*, 2003, pp. 41–54.
8. Bover-Cid, S. – Miguélez-Arrizado, M. J. – Becker, B. – Holzapfel, W. H. – Vidal-Carou, M. C.: Amino acid decarboxylation by *Lactobacillus curvatus* CTC273 affected by the pH and glucose availability. Food Microbiology, *25*, 2008, pp. 269–277.
9. Komprda, T. – Neznalová, J. – Standara, S. – Bover-Cid, S.: Effect of starter culture and storage temperature on the content of biogenic amines in dry fermented sausage poličan. Meat Science, *59*, 2001, pp. 267–276.
10. Lorenzo, J. M. – Cachaldora, A. – Fonseca, S. – Gómez, M. – Franco, I. – Carballo, J.: Production of biogenic amines "in vitro" in relation to the growth phase by *Enterobacteriaceae* species isolated from traditional sausages. Meat Science, *86*, 2010, pp. 684–691.
11. Bruna, J. M. – Hierro, E. M. – Hoz, L. – Mottram, D. S. – Fernández, M. – Ordóñez, J. A.: Changes in selected biochemical and sensory parameters as affected by the superficial inoculation of *Penicillium camemberti* on dry fermented sausages. International Journal of Food Microbiology, *85*, 2003, pp. 111–125.
12. Latorre-Moratalla, M. L. – Bover-Cid, S. – Vidal-Carou, M. C.: Technological conditions influence aminogenesis during spontaneous sausage fermentation. Meat Science, *85*, 2010, pp. 537–541.
13. Coïsson, J. D. – Cerutti, C. – Travaglia, F. – Arlorio, M.: Production of biogenic amines in "Salamini italiani alla cacciatora PDO". Meat Science, *67*, 2004, pp. 343–349.
14. Paulsen, P. – Bauer, F. – Vali, S.: Biogene Amine in Rohwürsten. 1. Methodische Aspekte zur Bestimmung biogener Amine. Fleischwirtschaft, *77*, 1997, pp. 450–452.
15. Drabik-Markiewicz, G. – Dejaegher, B. – De Mey, E. – Kowalska, T. – Paelinck, H. – Vander Heyden, Y.: Influence of putrescine, cadaverine, spermidine or spermine on the formation of N-nitrosamine in heated cured pork meat. Food Chemistry, *126*, 2011, pp. 1539–1545.
16. Ansorena, D. – Montel, M. C. – Rokka, M. – Talon, R. – Eerola, S. – Rizzo, A. – Raemaekers, M. – Demeyer, D.: Analysis of biogenic amines in northern and southern European sausages and role of flora in amine production. Meat Science, *61*, 2002, pp. 141–147.
17. Komprda, T. – Sládková, P. – Dohnal, V.: Biogenic amine content in dry fermented sausages as influenced by a producer, spice mix, starter culture, sausage diameter and time of ripening. Meat Science, *83*, 2009, pp. 534–542.
18. Bover-Cid, S. – Izquierdo-Pulido, M. – Vidal-Carou, M. C.: Changes in biogenic amine and polyamine cntents in slihtly fermented sausages manufactured with and without sugar. Meat Science, *57*, 2001, pp. 215–221.
19. Gençcelep, H. – Kaban, G. – Kaya, M.: Effects of starter cultures and nitrite levels on formation of biogenic amines in sucuk. Meat Science, *77*, 2007, pp. 424–430.
20. Pircher, A. – Bauer, F. – Paulsen, P.: Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. European Food Research and Technology, *226*, 2007, pp. 225–231.
21. Komprda, T. – Sládková, P. – Petirová, E. – Dohnal, V. – Burdychová, R.: Tyrosine- and histidine-decarboxylase positive lactic acid bacteria and enterococci in dry fermented sausages. Meat Science, *86*, 2010, pp. 870–877.
22. Garriga, M. – Aymerich, T: The microbiology of fermentation and ripening. In: Toldrá, F. (Ed.): Handbook of fermented meat and poultry. Ames :

Blackwell Publishing, 2007. 555 pp. ISBN 978-0-8138-1477-3

23. Bover-Cid, S. – Izquierdo-Pulido, M. – Vidal-Carou, M. C.: Effect of proteolytic starter cultures of *Staphylococcus* spp. on biogenic amine formation during the ripening of dry fermented sausages. International Journal of Food Microbiology, *46*, 1999, pp. 95–104.
24. Paulsen, P. – Grossgut, R. – Bauer, F. – Rauscher-Gabernig, E.: Estimates of maximum tolerable levels of tyramine content in foods in Austria. Journal of Food and Nutrition Research, *51*, 2012, pp. 52–59.
25. Yamanaka, H. – Shiomi, K. – Kikuchi, T.: Agmatine as a potential index for freshness of common squid. Journal of Food Science, *52*, 1987, pp. 936–938.
26. Ruiz-Capillas, C. – Moral, A.: Production of biogenic amines and their potential use as quality control indices for Hake (*Meluccius merluccius*, L.) stored in ice. Journal of Food Science, *66*, 2001, pp. 1030–1032.
27. Hernández-Jover, T. – Izquierdo-Pulido, M. – Veciana-Nogués, M. T. – Mariné-Font, A. – Vidal-Carou, M. C.: Biogenic amine and polyamine contents in meat and meat products. Journal of Agricultural and Food Chemistry, *45*, 1997, pp. 2098–2102.
28. Bover-Cid, S. – Miguélez-Arrizado, M. J. – Vidal-Carou, M. C.: Biogenic amine accumulation in ripened sausages affected by the addition of sodium sulphite. Meat Science, *59*, 2001, pp. 391–396.
29. Hui, Y. H. (Ed.): Handbook of food science, technology and engineering. Vol. 1. Boca Raton : CRC Press, 2006. 1 000 pp. ISBN 978-1574445510

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