Liquid Chromatographic Determination of Biogenic Amines in a Meat Product during Fermentation and Long-term Storage

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Abstract

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Liquid chromatographic procedures employing two derivatisation reagents, dansylchloride and *o*-phthaldialdehyde (OPA), were compared and applied for the determination of biogenic amines in meat products during fermentation and long-term storage. Both methods give similar results in terms of the detection limits, repeatability, recovery, and accuracy. Secondary amines (spermine and spermidine) do not react with *o*-phthaldialdehyde thus only their LC determination after derivatisation with dansylchloride is possible. Their contents during fermentation and/or long-term storage are nearly constant or slightly decrease, thus their determination in both cases is not necessary. LC procedure employing OPA derivatisation is faster, much simpler in terms of the pre-treatment of samples, and it can be fully automated using an intelligent auto sampler.

Keywords: biogenic amines; polyamines; HPLC determination; food; dry fermented sausage; ripening; storage

Biogenic amines represent a group of low-molecular-mass organic bases occurring in all organisms. Amines are formed and degraded during normal metabolic processes in living cells and therefore they are ubiquitous in animals, plants, and microorganisms (Halász *et al*. 1994). In foodstuffs, biogenic amines are usually generated by microbial decarboxylation of amino acids (Křížek & Kalač 1998). Thus, from histidine, tyrosine, tryptophane and phenylalanine, monoamines histamine, tyramine, tryptamine and 2-phenylethylamine, respectively, are formed and, similary, diamines putrescine and cadaverine are formed from ornithine and lysine, respectively. Putrescine is a precursor for the formation of polyamines spermidine and spermine (Bardócz 1993).

In dry fermented sausages, apart from the active growth of several microbial populations, acidification and proteolytic processes occurring during sausage fermentation make the environments particularly favourable for biogenic amine production (Brink *et al*. 1990; Hernandéz-Jover *et al*. 1996; Bover-Cid *et al*. 1999).

Excessive oral intake of biogenic amines, especially histamine and tyramine, can result in nausea, respiratory distress, heart palpitations, headache, and hyper- or hypotension (Silla-Santos 1996). Polyamines play an important role in cellular metabolism by stimulating cell, tissue and organs growth, and they also participate in the development of cancer (Bardócz 1995).

Thin-layer chromatography (TLC), gas chromatography (GC), capillary zone electrophoresis (CZE), and especially high-performance liquid chromatography (HPLC) have been proposed for the determination of amines. Sensitive chroma-

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tographic techniques including reversed-phase HPLC with fluorescence or UV monitoring of dansyl, benzoyl, 9-fluoromethyl chloroformate, N-hydroxysuccinimidyl 6-chinolyl carbamate or *o*-phthaldialdehyde (OPA) derivatives have been frequently used to detect amines. Ion-pair reversed-phase HPLC or ion exchange chromatography with OPA post column derivatisation are also often used. Mass spectrometry (MS) is now widely recognised as a very reliable detection and characterisation technique, especially if it is coupled with either GC or HPLC, particularly when two or more peaks of compounds of interest overlap (Seiler 1977; Harbone 1984; Smith & Davies 1987; Smith 1991, 1993; Careri *et al*. 1996; Bardelmeijer *et al*. 1998; Fernandes & Ferreira 2000).

MATERIAL AND METHODS

Material. Biogenic amines were determined during ripening and storage of the Czech dry fermented sausage "Herkules". The basic raw materials were: lean beef meat, lean pork and pork fat used in equal parts, nitrite salt mixture (2.5%), and sugars (1%). The best hygienic conditions including storage of all raw materials at –5°C for 48 h before the beginning of the production were applied. The batch was produced with the addition of the spicing mixture typical for the Czech dry fermented sausage "Herkules" and was applied according to the producer instructions. The starter culture containing *Pediococcus pentosaceus* and *Staphylococcus carnosus* was admixed in the amount of 50 g/100 kg of the product. Chopped and blended ingredients were stuffed into the cutisine casing of the diameter of 80 mm. Sausages were ripened for 3 weeks and consequently stored for 3 months at room temperature. Samples were analysed in a fortnight intervals (except the first one taken after 1 week of storage).

A sample of sausage was homogenised in a Moulinex blender (Moulinex, France), an aliquot (10 g ± 1 mg) was then put into a 85 ml test tube, 0.5 ml of an internal standard (1,7-diaminoheptane; concentration 1 mg/ml) was added and the sample was extracted for 2 min with 15 ml of 5% trichloroacetic acid (TCA) using disintegrator Heidolph Diax 900 with a model number 1624 rotor, radius 134 mm (Heidolph, Germany) at speed 3 for 2 min. The suspension was centrifuged at 3000 rpm for 10 min at 4°C (Hettich Universal 32R, Germany). The supernatant was filtered through a paper filter and the solid residue was repeatedly extracted as given above (usually twice). The combined extracts were made up to 50 ml by deionised water and filtered through a disposable nylon membrane filter (13 mm, 0.45 μm, Chromatography Research Supplies, Addison, USA).

Equipment. Biogenic amines were separated using a liquid chromatograph HP 1100 (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump (G1311A), a vacuum degasser (G1322A), an auto sampler (G1313A), a UV/VIS detector with variable wavelength (G1314A), and a fluorescence detector (G1321A). The separation after *o*-phthaldialdehyde (OPA) derivatisation was performed on a reversed-phase column Zorbax Eclipse XDB C8 (150 mm × 4.6 mm, particle size 5 μm) equipped with a guard column Meta Guard Inertsil C18 (30 mm × 4.6 mm, particle size 5 μm). A gradient elution (see Table 1 for the elution profile) was used with a mobile phase consisting of 100 mM acetate buffer (A; pH 5.8) and acetonitrile (B, ACN) at a flow rate 0.6 ml/min and

Table 1. HPLC elution program

Time (min)	OPA		Time (min)	DnsCl	
	A (%)	ACN (%)		H_2O (%)	ACN (%)
0	60	40	0	35	65
10	60	40	1	35	65
15	40	60	10	20	80
20	35	65	12	10	90
27	23	77	16	0	100
			23	0	100

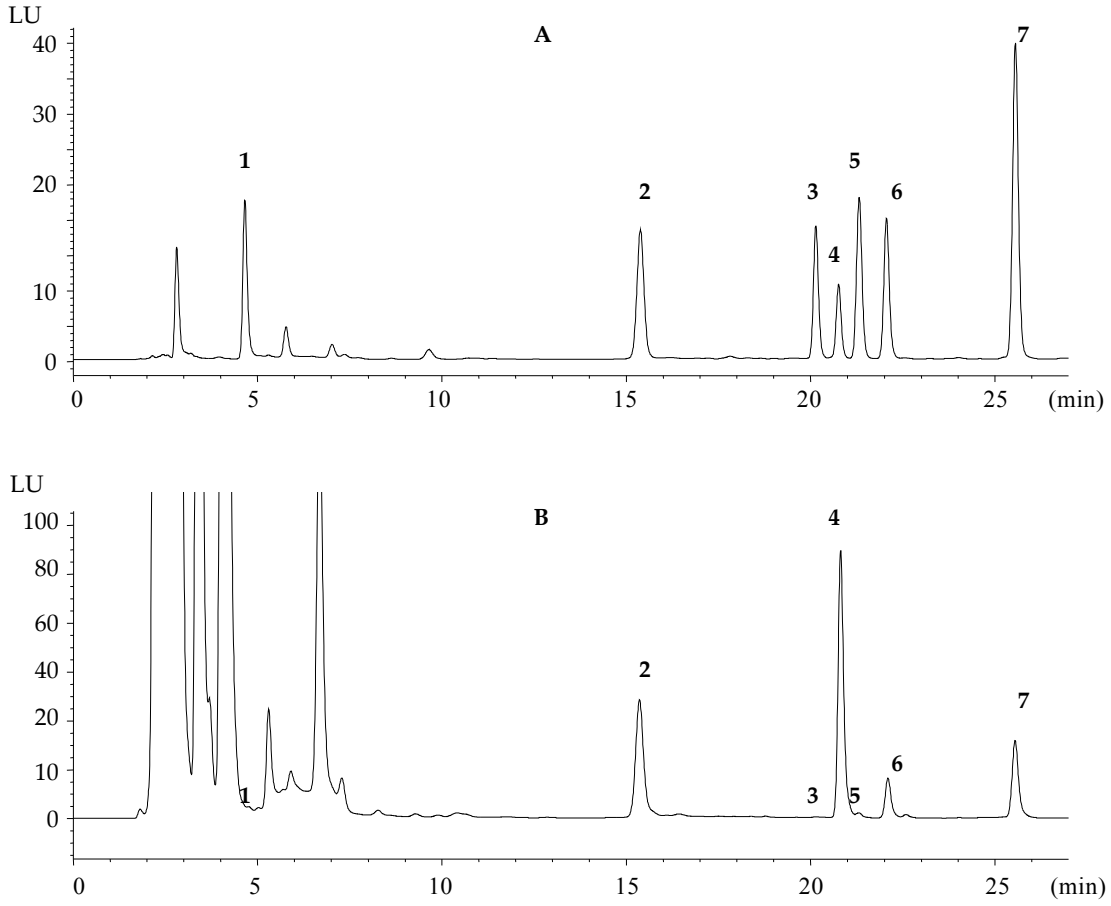
A = 100mM acetate buffer (pH 5.8); ACN = acetonitrile, flow rate 0.6 ml/min and 0.8 ml/min, respectively

at room temperature. The eluted OPA derivatives were detected by fluorescence using 330 nm and 440 nm as excitation and emission wavelengths, respectively.

The separation after dansyl chloride derivatisation (DnsCl) was carried out by gradient elution with H_2O/ACN (see Table 1 for the elution profile) on a Zorbax Eclipse XDB C18 column (150 mm × 4.6 mm, particle size 5 μm) with a guard column Meta Guard ODS-2 (30 mm × 4.6 mm, particle size 5 μm) at the flow rate of 0.8 ml/min using a photometric UV/VIS detector at 254 nm. The pH of the acetate buffer was adjusted with the stepwise addition of NaOH using pH meter InoLab pH Level 1 (WTW, Weilheim, Germany).

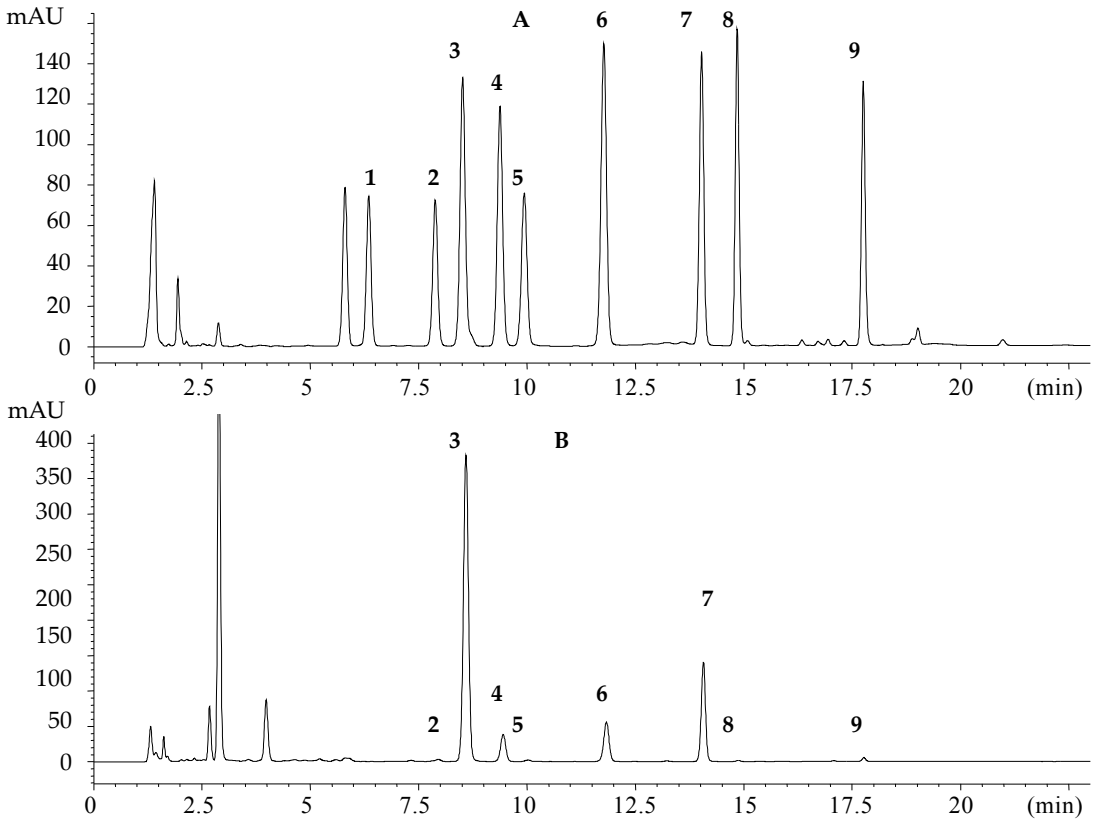
Reagents and standards. Histamine (HI), tyramine (TY), tryptamine (TRP), putrescine (PUT), 2-phenylethylamine (PEA), cadaverine (CAD), spermidine (SPD), and spermine (SPM) as hydrochloride salts, *o*-phthaldialdehyde (> 99%), and 1,7-diaminoheptane (1,7-DH) were obtained from Sigma-Aldrich (Steinhein, Germany). An individual standard stock solution of each amine was prepared in water Milli-Q RG and stored in darkness at 4°C; the concentration of each solution was 1 mg/ml. Working standard solutions of the concentration of 0.01 mg/ml were prepared weekly since some amines decompose. All results were corrected on the basis of their purity and referred to the free base.

Dansyl chloride (5-dimethylaminonaphthalene-1-sulphonylchloride, > 98%), trichloroacetic acid (> 99%), boric acid (> 99.5%), acetonitrile (ACN) of HPLC grade, and ethanol (99.5%) were obtained from Merck (Darmstadt, Germany). Acetic acid, anhydrous sodium carbonate and diethyl ether



1 – histamine, 2 – tyramine, 3 – tryptamine, 4 – putrescine, 5 – 2-phenylethylamine, 6 – cadaverine, 7 – 1,7-diaminoheptane. For chromatographic conditions see chapter Material and Methods

Figure 1. HPLC chromatograms of OPA derivatives of biogenic amines. Standard solution of the concentration or 0.01 mg/ml (A) and extract of sausage (B)



1 – tryptamine, 2 – 2-phenylethylamine, 3 – putrescine, 4 – cadaverine, 5 – histamine, 6 – 1,7-diaminoheptane, 7 – tyramine, 8 – spermidine, 9 – spermine. For chromatographic conditions see chapter Material and Methods

Figure 2. HPLC chromatograms of DnsCl derivatives of biogenic amines. Standard solution (A) and extract of sausage (B)

were from Pliva-Lachema (Brno, Czech Republic). Demineralised water (AquaDem-2, AquaOsmotic, Tišnov, Czech Republic) purified further with a Milli-Q RG system (Millipore, Bedford, USA) was used for the preparation of solutions and for the dilution of all solvents.

o-phthaldialdehyde (OPA) derivatisation. Derivatisation reagent (OPA) was prepared according to Lindroth and Mopper (1979) by dissolving 27 mg of *o*-phthaldialdehyde in 0.5 ml of ethanol (99%), adding 20 µl 2-mercaptoethanol, > 98%, and making up to the volume of 5 ml with 0.4M borate buffer (pH 9.5 adjusted by 1M NaOH). 0.5 µl of the extract was mixed with 2.5 µl of OPA and after the reaction time (2 min), the mixture was injected onto the chromatographic column. Typical chromatograms of OPA derivatives of the standard and of the extract of sausage are shown in Figure 1.

Dansyl chloride (DnsCl) derivatisation. Derivatisation of biogenic amines was performed by a modification of the methods described in articles Moret *et al*. (1992), Eerola *et al*. (1993) and Vale & Gloria (1997). 1 ml of the extract (or standard) was mixed with 0.5 ml of saturated solution of Na_2CO_3, 1 ml of DnsCl (5 mg of dansyl chloride per 1 ml acetone) was added and the mixture was shaken for 1 minute (MS2 Minishaker, IKA Werke, Germany). Derivatisation proceeded for 1 hour in dark. After derivatisation, 250 µl of ammonia was added to remove the excess of DnsCl. No interference was observed since the reaction product is eluted before other biogenic amines. Hydrophobic derivates of amines were extracted by diethyl ether (3 × 1 ml) after 30 min, derivatives of amino acids remained in the water phase. The organic phase was evaporated to dryness under nitrogen, the solid residue was dissolved in 0.5 ml of acetonitrile (standard in 1 ml acetonitrile), and the solution was filtered through a nylon membrane filter 0.45 µm and injected onto the chromatographic

column. The volumes of the real samples injected were modified; the volume of the standard was 10 µl. Chromatograms of DnsCl derivatives are given in Figure 2.

Identification. The separated amines were identified by comparison of their retention times with those of the standards. As regards OPA derivatives, they were also identified by comparison of the absorption spectra of the standards stored in the user's library.

Quantification. The whole procedure involves multiple steps, and for this reason the use of an internal standard (IS) is desirable. The concentration of biogenic amine in the sample (c_x) was calculated as follows:

$$c_x = RF_x \times (c_{IS} \times A_x)/A_{IS}$$

where: RF_x – response factor of the amine ($RF_x = A_{IS}/A_x \times c_x/c_{IS}$)
A_{IS} – peak area of the internal standard
A_x – peak area of biogenic amine
c_{IS} – concentration of the internal standard

RESULTS AND DISCUSSION

Selection of the suitable extraction solvent

Two solvents were studied in view of their suitability for the amine extraction from sausages: 5% trichloroacetic acid (TCA) and 0.4M perchloric acid ($HClO_4$) in duplicates and triplicates. The extraction efficiency was compared using the results and the peak areas of OPA derivatives (Table 2). The best recovery for this type of material was observed using extraction with TCA in triplicates. Clean up of DnsCl derivatives was examined using extraction with diethyl ether in duplicates and triplicates. The recovery was higher by 7–27% after the third extraction than after the second one. The time stability of the extracts was verified after several months storage at –18°C. After six months we observed neither quantitative nor qualitative changes.

Method validation

In this work the amines were determined by HPLC using two methods based on pre-column derivatisation and a polarity gradient. In both methods, we used the reversed-phase column. Without derivatisation the amines would elute as broad peaks as shown by fluorescence detection. UV absorbance detection is only possible for the heterocyclic and aromatic amines, therefore derivatisation is necessary for the detection of aliphatic amines and for increased sensitivity. Pre-column derivatisation was chosen since it allows the secondary product separation on the column

Table 2. Comparison of extraction recovery with a of 5% TCA or 0.4M $HClO_4$ in duplicates and triplicates in the sample of "Herkules" salami after three weeks of storage, and the efficiency of extract purifacation after extraction with diethyl ether in duplicates and triplicates for the same sample at the end of storage as compared with the peak areas (in milliabsorbance units per second – mAU/s)

Amine	Number of extractions						Increase of recovery (%)
	2	3	2	3	2	3	
	5% TCA		0.4 M $HClO_4$		diethyl ether		
	peak areas (mAU/s)						
Histamine	14.6	15.9	15.4	15.3	46.5	59.6	22.0
Tyramine	848	1 010	903	936	4 040	4 990	19.1
Tryptamine	4.7	5.4	4.3	4.7	19.5	21.9	11.0
Putrescine	1 460	1 720	1 520	1 510	12 000	14 300	16.0
2-Phenylethylamine	25.4	29.7	27.6	30.1	52	56	7.1
Cadaverine	226	270	236	237	1 030	1 240	17.3
Spermidine					91.3	126	27.4
Spermine					688	856	19.6
1,7-Diaminoheptane	453	486	467	469	1 190	1 490	20.4

Table 3. Parameters of calibration curves ($y = ax + b$), correlation coefficients (R^2) and detection limits (LOD for 3.S/N) for concentration range C < > (ng injected, OPA derivatives 0.5 µl, DnsCl derivatives 10 µl)

Amine	OPA					DnsCl				
	C < > (ng)	*a*	*b*	R^2	LOD (ng)	C < > (ng)	*a*	*b*	R^2	LOD (ng)
Histamine	0.31–30.61	65.602	25.979	0.9990	0.0026	1.5–377.4	17.215	–30.044	0.9998	0.0012
Tyramine	0.44–43.53	60.331	20.522	0.9992	0.0037	2.0–493.7	13.005	–35.327	0.9998	0.0015
Tryptamine	0.42–41.71	45.351	17.376	0.9992	0.0037	2.0–509.1	6.8742	–14.419	0.9998	0.0028
Putrescine	0.29–58.83	33.36	26.845	0.9994	0.0046	1.4–342.1	21.625	–43.029	0.9990	0.0009
2-Phenyl-ethylamine	0.39–38.86	63.008	32.575	0.9990	0.0030	1.9–480.4	7.6724	–19.442	0.9999	0.0025
Cadaverine	0.30–29.8	68.196	26.717	0.9995	0.0027	1.5–364.7	17.215	–30.044	0.9998	0.0011
Spermidine						1.4–356.5	17.551	–64.873	0.9998	0.0011
Spermine						1.5–363.2	14.892	–70.514	0.9995	0.0013

avoiding loss during the analysis. A disadvantage of the post-column derivatisation would be a prolongation of the separation time (80–130 min) and the peak broadening (Badelmeijer *et al*. 1998). The peaks of the biogenic amines were satisfactorily resolved and no interfering peaks appeared.

To control the linear range of UV-VIS and FLD detector, the calibration curves between the responses of the injected standard solutions (peak areas) and the corresponding amine concentrations were measured. The calibration curves were linear in the range of 0.3–54.7 ng in the injected volume (0.5 µl) for OPA derivatives, and 1.4–509.1 ng (injected volume 10 µl), respectively, for DnsCl derivatives. The concentration ranges were typical for amine concentrations detected in the sausage samples. Linear least-squares regression was used to calculate the slopes, intercepts, and correlation coefficients. The data on the linearity and detection limits (LOD for 3 × S/N) are given in Table 3, a linear relationship with $R^2 > 0.999$ was always obtained.

The standard solution was derivatised and the mixture was injected ten times to evaluate the repeatability of the chromatographic system. The repeatability of the complete analytical pro-

Table 4. Response factors (RF) and amine concentrations (± standard deviation, $n = 10$) of OPA and DnsCl derivatives. Results are means for ten replicates of the same sample. RSD = relative standard deviation ($n = 10$)

Amine	OPA derivatives				Dansyl derivatives			
	instrument		method		instrument		method	
	RF[a]	RSD (%)	C (mg/kg)	RSD (%)	RF[a]	RSD (%)	C (mg/kg)	RSD (%)
Histamine	1.515 ± 0.037	2.5	2.52 ± 0.10	3.90	1.398 ± 0.005	0.3	2.34 ± 0.05	2.30
Tyramine	1.656 ± 0.026	1.6	0.67 ± 0.02	3.64	1.012 ± 0.017	1.6	1.90 ± 0.06	3.19
Tryptamine	2.166 ± 0.025	1.1	0.44 ± 0.01	3.17	1.935 ± 0.006	0.3	0.21 ± 0.01	3.48
Putrescine	2.733 ± 0.034	1.2	0.94 ± 0.06	6.43	0.627 ± 0.002	0.3	5.16 ± 0.35	6.69
2-Phenyl-ethylamine	1.553 ± 0.016	1.0	0.04 ± 0.00	4.97	1.557 ± 0.005	0.4	0.63 ± 0.02	2.65
Cadaverine	1.328 ± 0.013	1.0	0.22 ± 0.01	3.34	0.718 ± 0.002	0.3	1.81 ± 0.06	3.19
Spermidine					0.846 ± 0.002	0.3	1.90 ± 0.07	3.93
Spermine					0.994 ± 0.002	0.2	12.52 ± 0.48	3.81

[a]RF = [(I.S. area/standard amine area) × (standard amine concentration)/I.S. concentration]

Table 5. Recovery (%)

Amine	DnsCl	OPA	DnsCl	OPA
	1 mg/kg		2 mg/kg	
Histamine	117	72	138	79
Tyramine	91	93	95	94
Tryptamine	78	78	76	78
Putrescine	94	94	98	95
2-Phenylethylamine	91	91	89	90
Cadaverine	95	92	97	93
Spermidine	82		85	
Spermine	96		87	

cedure was examined using a sample of sausage with a low amine content. Five aliquots of the same sample were analysed using the procedure proposed. Mean values, standard deviations (SD), and relative standard deviations (RSD) are given in Table 4. These values are comparable with the data of Petridis and Steinhart (1995): RSD = 1.27% (PEA)–3.52% (TY) for the OPA derivatives, and of Eerola *et al*. (1993): RSD = 3.9% (TRP)–18.1% (PUT) for the dansyl derivatives.

The recovery of amines during extraction and purification was investigated by a standard addition technique at 2 levels (1 and 2 mg/kg) in duplicates. To calculate the recovery, the concentrations determined before and after the standard addition were compared (Table 5). These values of recovery are also comparable with the data of Petridis and Steinhart (1995): 94% (HI)–103% (PUT) for the OPA derivatives, and of Eerola *et al*. (1993): 84% (TRP)–104% (PEA).

Control of the amine content during ripening and storage

The above-mentioned method was applied for the determination of biogenic amine content during fermentation and storage of the dry fermented salami "Herkules". Progress in the amine contents determined as OPA derivatives (HI, TY, TRP, OUT, PEA, CAD) and DnsCl derivatives (SPD, SPM) is shown in Table 6. Quantitatively the most important biogenic amines in the present experiment were putrescine, tyramine, and spermine during ripening, and putrescine, tyramine and cadaverine during storage. Tyramine was also the most abundant amine in three of the four types of northern and southern European sausages in the experiment of Ansorena *et al*. (2002). Similar results regarding tyramine and cadaverine were also reported by Bover-Cid *et al*. (1999, 2001) as far as tyramine and putrescine are concerned. Tyramine is most toxic, toxicological level being 100–800 mg/kg (Silla-Santos 1996). From this viewpoint, the

Table 6. Biogenic amine content during "Herkules" ripening and storage

Week	Biogenic amine (mg/kg DM) (mean ± standard error of the mean; n = 2)							
	HI	TY	TRP	PUT	PEA	CAD	SPD	SPM
0	2 ± 0.4	n.d.	n.d.	n.d.	n.d.	n.d.	4 ± 0.1	27 ± 1.8
1	1 ± 0.2	54 ± 2.4	1 ± 0.1	121 ± 4.0	n.d.	4 ± 0.0	3 ± 0.1	26 ± 1.3
2	3 ± 1.1	91 ± 1.0	3 ± 0.0	187 ± 4.6	1 ± 0.0	9 ± 0.2	3 ± 0.2	24 ± 4.2
3	2 ± 0.1	123 ± 1.5	3 ± 0.1	247 ± 0.6	1 ± 0.1	15 ± 0.1	4 ± 0.2	30 ± 0.1
4	3 ± 0.0	120 ± 6.0	1 ± 0.1	260 ± 0.9	1 ± 0.1	18 ± 0.9	3 ± 0.0	27 ± 0.0
6	2 ± 0.8	158 ± 0.4	1 ± 0.0	360 ± 10.2	4 ± 0.1	32 ± 0.0	2 ± 0.2	19 ± 3.4
8	1 ± 0.0	204 ± 8.6	1 ± 0.1	408 ± 0.4	5 ± 0.4	43 ± 9.1	2 ± 0.4	12 ± 6.7
10	2 ± 0.2	187 ± 5.0	n.d.	425 ± 0.9	5 ± 0.1	35 ± 0.7	2 ± 0.2	10 ± 4.2
12	1 ± 0.0	228 ± 8.7	1 ± 0.0	515 ± 8.5	5 ± 0.0	40 ± 1.2	2 ± 0.1	14 ± 0.8
14	10 ± 0.6	469 ± 16.1	1 ± 0.8	820 ± 6.1	8 ± 1.0	46 ± 0.9	2 ± 0.3	16 ± 0.8
16	7 ± 0.4	359 ± 1.4	1 ± 0.9	601 ± 7.2	8 ± 0.2	36 ± 0.7	2 ± 0.6	11 ± 5.6

n.d. – not detected

"Herkules" – sausage in the present experiment should be considered hazardous, especially when simultaneously high concentrations of putrescine, a known tyramine toxicity potentiator (Bover-Cid *et al*. 2000), were found in this product. Moreover, Til *et al*. (1997) found acute and subacute toxicity of putrescine and cadaverine to be at the same level as tyramine (No-Observed-Adverse-Effect-Level of either amine 83 mg/kg body weight/day). A significant increase ($P < 0.01$) of the content during ripening and storage was found only for tyramine and putrescine. The contents of secondary amines (spermine and spermidine) are nearly constant or slightly decreased during fermentation and/or long-term storage.

Conclusions

Liquid chromatographic procedures employing two derivatisation reagents, dansylchloride and *o*-phthaldialdehyde, were compared and applied for the determination of biogenic amines in meat products during fermentation and long-term storage. Both methods gave similar results in terms of the limits of detection, repeatability, recovery, and accuracy. Secondary amines (spermine and spermidine) do not react with *o*-phthaldialdehyde and thus their LC determination is only possible after derivatisation with dansylchloride. Their contents during fermentation and/or long-term storage is nearly constant or slightly decreased, thus their determination in both cases is not necessary but due to their activity, the control should not be omitted. LC procedure employing OPA derivatisation is faster, much simpler in terms of the pre-treatment of samples, and it can be fully automated using an intelligent auto sampler.

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Souhrn

Smělá D., Pechová P., Komprda T., Klejdus B., Kubáň V. **(2003): Chromatografické stanovení biogenních aminů v trvanlivých salámech během fermentace a skladování.** Czech J. Food Sci., **23**: 167–175.

Byly porovnány dvě chromatografické metody stanovení biogenních aminů po jejich derivatisaci *o*-ftaldialdehydem (OPA) a dansyl chloridem a obě metody byly použity pro kontrolu jejich obsahu v masném výrobku. Oběma metodami byly získány uspokojující výsledky týkající se rozsahu linearity, detekčních limitů, opakovatelnosti i návratnosti pro tento typ matrice. Sekundární aminy (spermin a spermidin) nereagují s *o*-ftaldialdehydem, proto je možné jejich stanovení pouze po derivatisaci dansyl chloridem. V případě procesu zrání a skladování salámů zůstává obsah sekundárních aminů konstantní nebo se naopak snižuje, proto není nutné sledovat jejich obsah během celého procesu. Příprava vzorků pro chromatografické stanovení aminů po derivatisaci OPA je výrazně jednodušší, vyžaduje podstatně méně času a umožňuje plnou automatizaci při použití inteligentního autosampleru.

Klíčová slova: biogenní aminy; polyaminy; HPLC; potraviny; trvanlivé salámy; fermentace; skladování

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