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Concentrations of seven biogenic amines in sauerkraut

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Abstract

Biogenic amines were determined in 121 sauerkraut samples as *N*-benzamides by micellar electrokinetic capillary chromatography. The samples were divided into four groups: from Czech manufacturers, Austrian manufacturers, household-prepared and sterilized with brine in jars. Mean concentrations were 174, 146 and 50 mg kg^{-1} for tyramine, putrescine and cadaverine, respectively. However, very wide variations occurred. The lowest concentrations were found in household-prepared sauerkraut. Histamine levels were below 2 mg kg^{-1} in 44% of samples and above 10 mg kg^{-1} in only 19% of samples. The concentrations of tryptamine, spermidine and especially of spermine were low. No significant correlations were observed between sauerkraut quality parameters and amine concentrations. From the nutritional point of view, high tyramine levels should be taken under consideration. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Biogenic amines (BAs) form a group of natural biologically-active compounds occurring in numerous foods. They arise mainly from microbial decarboxylation of the corresponding amino acids. The most common monoamines, histamine (HI), tyramine (TY) and tryptamine (TR), and the diamines (or polyamines), putrescine (PUT) and cadaverine (CAD), are formed from histidine, tyrosine, tryptophan, ornithine and lysine, respectively. Polyamines, spermidine (SPD) and spermine (SPM) arise from putrescine.

A scale of symptoms occurs following an excessive oral intake of the BAs. They cause mainly psychoactive and/or vasoactive effects. Psychoactive amines affect the neural transmitters. The vasoactive ones act either directly or indirectly on the vascular system as vasoconstrictors (e.g. TY) or vasodilators (e.g. HI), but other amines, namely PUT, may potentiate these properties. The deleterious effects depend strongly on the efficiency of detoxification, which may vary considerably among individuals and are affected by several factors. Usual intakes of the BAs are metabolized in the intestinal tract by a fairly efficient detoxification system based on the activities of monoamine oxidase (MAO, EC 1.4.3.4) and diamine oxidase (DAO, EC 1.4.3.6).

However, polyamines SPD, SPM and PUT are known to fulfil an array of roles in cellular metabolism. One of the most important processes are metabolic signals stimulating growth of cells, tissues and organs (Bardócz, 1993; Bardócz, Grant, Brown, Ralph & Pusztai, 1993). Thus, their intake in foods may be desirable under some physiological conditions. Nevertheless, some risk of *N*-nitrosocompound formation should be taken into consideration (Shalaby, 1996).

Numerous reviews were published in the 1990s on occurrence and formation of BAs in different foods and their roles in nutrition (Beutling, 1996; Brink, Damink, Joosten & Huis in't Veld, 1990; Davídek & Davídek, 1995; Halász, Baráth, Simon-Sarkadi & Holzapfel, 1994; Křížek & Kalač, 1998; Shalaby, 1996; Silla Santos, 1996; Slorach, 1991; Stratton, Hutkins & Taylor, 1991). The highest levels of HI have been found in scombroid fish, while those of TY in some ripened cheeses. Medium levels have been usually observed in fermented products, including sauerkraut.

Sauerkraut, shredded white cabbage preserved by lactic fermentation, has been popular in many European countries due to its sensorial properties and favourable nutritional value. It has been the most used vegetable during the cold season in the Czech Republic.

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Data given in Table 1, on contents of the BAs in sauerkraut, and the valuable review of Buckenhüskes, Sabatke, and Gierschner (1992), show great ranges of concentrations. Thus, sauerkraut could represent an important intake of the amines, at least in some individuals.

The objective of the present work was to determine concentrations of seven BAs in different sauerkraut types and to look for any correlations with common quality criteria.

2. Materials and methods

2.1. Sampling

In total, 121 samples, taken from February 1997 to January 1999, were divided into four groups:

- 53 samples from 12 Czech commercial manufacturers, packed in plastic sachets (usually 500 g) without pasteurization
- 10 samples from three Austrian manufacturers, pasteurized, packed similarly in plastic sachets,
- 29 samples of household-prepared sauerkraut,
- 29 samples of sauerkraut in brine, sterilized in jars from 16 Czech manufacturers.

All market sauerkrauts were taken from retail shops. All Czech sauerkrauts were prepared by the spontaneous fermentation in released cabbage juice with added common salt and spices, preferably caraway. Shredded onions were often added in household-prepared sauerkrauts. No inoculants of lactic acid bacteria were applied. Unfortunately, no information on technology of the Austrian manufacturers was available.

2.2. Analytical methods

Sauerkraut was cleared of free brine or juice. A water extract for the determination of quality criteria was prepared from 100 g of sauerkraut and 900 ml of water standing overnight and filtered. The acidic extract for BAs determination was prepared from 20 g of sauerkraut with about 75 ml of 0.6 M $HClO_4$ and shaken in a closed Erlenmeyer flask for one hour. The mixture was filtered through a filter paper, washed with perchloric acid and volume was adjusted to 100 ml. Prior to the BAs determination (maximally 2 weeks) the extracts were stored in a refrigerator. All analyses were done in duplicates and mean values were used. All chemicals were of analytical grade.

Water extract pH value was measured and total acidity and alpha amino group contents were determined by potentiometric titration to pH 8.3 using 0.05 M NaOH and neutralised formaldehyde. Total acidity was expressed as mg NaOH per 100 g of sauerkraut, total alpha amino groups as mg of -NH_2 per 100 g of sauerkraut. Ammonia content was determined by Conways microdiffusion method.

Volatile alcohols (C_1–C_4) were determined in water extract by a gas chromatographic method described by Špička (1995) using Varian 3300 (Varian Techtron, Walnut Creek, CA) with a glass packed column 2000/2 mm filled with 0.3% Carbowax 20M and 0.1% H_3PO_4 on Carbopack C 60/80 mesh and temperatures 90, 200 and 200°C for column, injection and flame ionization detector, respectively.

Lactic acid and volatile fatty acids (C_1–C_4) were determined isotachophoretically using Agrofor apparatus (JZD Odra, Krmelín, Czech Rep.) according to a method developed by the manufacturer of the apparatus: capillary 300/0.4 mm, electric current 120μA/60 μA,

Table 1
Literature data on biogenic amine concentrations in sauerkraut[a]

Authors	Year	Country	*n*	Biogenic amines (mg kg^{-1})				
				HI	TY	CAD	PUT	SPD
Mayer and Pause	1972	Switzerland	2	(7;200)	(20;95)	(3;30)	(1;40)	–
Taylor, Leatherwood and Lieber	1978	CA, USA	50	5.1 (0.9–13.0)	–	–	–	–
Künsch et al.	1989	Switzerland	12	29 (2–63)	43 (7–66)	28 (1–81)	174 (13–443)	–
Brink et al.	1990	Netherlands	8	38 (1–104)	75 (2–192)	73 (1–311)	154 (6–550)	–
Buckenhüskes et al.	1992	Germany	8	44 (12–78)	41 (2–82)	62 (28–121)	131 (14–251)	–
Halász et al.	1994	Hungary	8	(42–52)	–	(53–71)	(100–200)	(3–8)
Ziegler, Hahn and Wallnöfer	1994	Germany	3 Bags	56 (32–79)	89 (58–139)	24 (14–36)	222 (195–272)	6.4 (5.1–7.2)
			3 Cans	–	24.7 (19–28)	4.0 (3.0–5.1)	90 (72–103)	6.4 (6.1–6.8)

[a] Data are mean values; ranges are given in parentheses.

Table 2
Statistical characteristics of sauerkraut samples divided into four groups

Parameter	All samples $n=121$				Czech manufacturers $n=53$				Austrian manufacturers $n=10$				Household $n=29$				Sterilized $n=29$			
	$\bar{x}$	STD	x_{min}	x_{max}	$\bar{x}$	STD	x_{min}	x_{max}	$\bar{x}$	STD	x_{min}	x_{max}	$\bar{x}$	STD	x_{min}	x_{max}	$\bar{x}$	STD	x_{min}	x_{max}
Amines (mg kg^{-1})																				
HI	7.8	21.6	ND	229	12.1	31.6	ND	229	2.1	2.4	ND	8.0	4.6	6.8	ND	32.4	4.9	6.4	ND	26.4
TY	174	167	ND	951	235	213	ND	951	130	71.3	14.0	214	117	113	ND	384	134	90.4	26.3	345
PUT	146	99.0	2.8	529	181	108	2.8	529	179	80.2	51.0	295	87.3	72.2	4.3	260	132	81.5	18.4	359
CAD	50.0	46.2	ND	293	64.8	56.8	1.9	293	43.4	21.0	19.3	77.4	29.8	23.0	ND	82.7	45.5	40.1	6.9	167
TR	5.1	8.7	ND	37.5	4.6	9.0	ND	36.5	2.4	3.2	ND	7.7	4.7	7.9	ND	28.1	7.2	10.2	ND	37.5
SPD	8.2	6.6	ND	47.0	8.2	7.3	ND	47.0	6.5	5.5	ND	16.9	10.2	7.5	ND	28.3	6.8	4.0	ND	15.2
pH	3.54	0.16	3.27	4.39	3.55	0.16	3.32	4.39	3.62	0.16	3.44	3.96	3.55	0.16	3.29	3.93	3.49	0.15	3.27	3.81
Total acidity (mg NaOH 100 g^{-1})	505	143	180	1080	477	150	180	1080	486	94	310	630	566	128	225	780	503	144	240	840
Lactic acid (% w/w)	1.29	0.37	0.43	2.16	1.29	0.38	0.43	2.06	1.35	0.38	0.82	1.84	1.48	0.27	0.79	2.16	1.07	0.33	0.57	1.81
Acetic acid (% w/w)	0.33	0.14	ND	0.78	0.30	0.11	ND	0.52	0.30	0.06	0.23	0.37	0.31	0.09	0.17	0.45	0.44	0.20	0.25	0.78
Alpha-amino groups (mg 100 g^{-1})	45.9	20.4	10.0	164	42.6	15.2	17.0	79.0	54.6	19.9	29.0	97.0	56.9	26.3	31.0	164	38.0	17.4	10.0	90.0
Ammonia (mg 100 g^{-1})	22.4	10.0	5.0	60.0	21.8	8.2	9.0	39.0	23.8	5.9	19.0	39.0	20.8	11.8	5.0	60.0	24.7	11.9	5.0	49.0
Ethanol (% w/w)	0.28	0.24	0.02	1.58	0.34	0.31	0.02	1.58	0.16	0.08	0.10	0.34	0.29	0.22	0.08	0.99	0.20	0.09	0.10	0.57

leading electrolyte 240 mg (ε-aminocaproic acid, 10 ml 0.1 M HCl and 5 ml 0.2% (w/w) hydroxyethylcellulose filled up to 100 ml with water and terminating electrolyte 115 mg caproic acid in 100 ml of water.

Repeatability of the analytical procedures was tested by six parallel analyses of a sauerkraut sample. Relative standard deviations were 0.12% at mean pH value 3.52, and 0.9, 1.5, 2.3, 3.5, 5.6 and 3.0% at mean concentrations 632, 35.0, 11.8 mg 100 g^{-1}, 0.79, 1.44 and 0.35% for total acidity, amino groups, ammonia, ethanol, lactic acid and acetic acid, respectively.

Seven observed BAs were determined as *N*-benzamides by a method of micellar electrokinetic capillary chromatography, described in detail by Křížek and Pelikánová (1998), using Spectraphoresis 2000 (Thermo Separation Products, Fremont, CA). The detection limits were 1.0, 1.3, 1.4, 1.4, 2.1, 2.1 and 3.5 mg kg^{-1} of sauerkraut for SPD, TR, CAD, SPM, PUT, HI and TY, respectively.

Repeatability of the analytical procedure was tested similarly as for quality criteria. Relative standard deviations were 11.2, 7.8 and 7.1% at mean concentrations 93.3, 43.3 and 53.3 mg kg-1 for TY, PUT and CAD, respectively.

2.3. *Statistical methods*

Statistical data were obtained using Microsoft Excel 5.0.

3. Results and discussion

Summarized data on BAs concentrations and quality parameters are given in Table 2. The determined sauerkraut quality parameters were relatively stable. The only sample of poor sensorial properties showed a pH value exceeding 4.0 with an extreme histamine concentration of 229 mg kg^{-1}. Acetic acid was found as the only volatile fatty acid. Similarly, within volatile alcohols only ethanol was present in all samples, and traces of 2-propanol were found in several samples. No significant correlation was observed between the individual quality parameters and either the individual amine or sum of BAs concentrations.

Prior to discussion of the observed BAs concentrations, the recommended maximal BAs levels in foods should be mentioned. Nout (1994) proposed as acceptable levels for fermented foods, 50–100, 100–800, 30 or 100–200 mg kg^{-1} for HI, TY, 2-phenylethylamine or total, respectively. Künsch, Schärer and Temperli (1989) recommended for good quality sauerkraut maximal values of 10, 20, 50, 25 and 5 mg kg^{-1} for HI, TY, PUT, CAD and 2-phenylethylamine, respectively.

Three amines, TY, PUT and CAD, were observed in virtually all samples. However, variations of their contents were large, and maximal values were often higher than those reported in Table 1. Great differences may be explained mainly by different microflora during spontaneous fermentation, though not only different

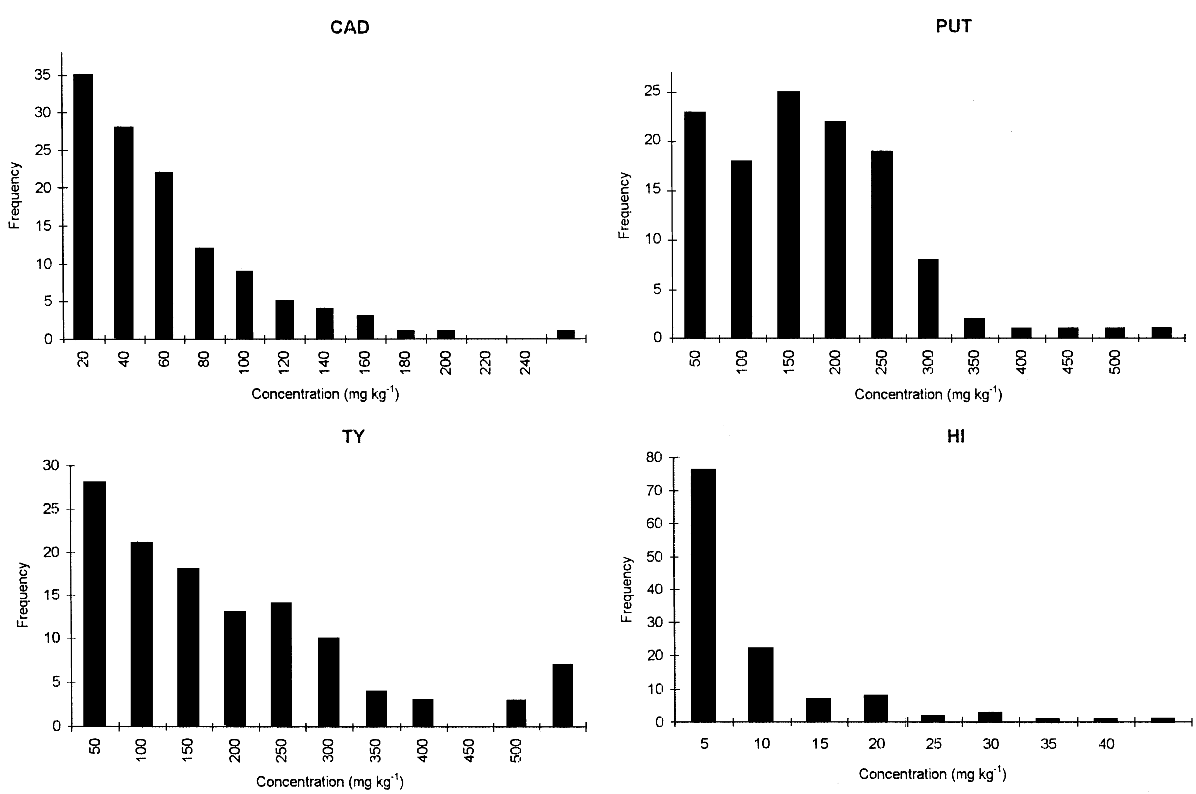

Figure 1. Histograms of four amines.

species, but also different strains may vary in magnitude of three orders in their ability to produce BAs. Thus, many factors affect BAs formation, such as temperature, pH value changes, oxygen access or sodium chloride content (Shalaby, 1996; Silla Santos, 1996; Straub, Kicherer, Schilcher & Hammes, 1995).

Tyramine concentrations given in Table 2 and their frequency given in Fig. 1 may be considered as commonly high, though a single TY intake of 10–80 mg may cause swellings and, over 100 mg, migraine (Askar and Treptow, 1986). For patients using MAO inhibitors, TY intake should not exceed 6 mg during 4 h.

Only 9.9% of samples had TY levels below 20 mg kg^{-1}, while 47.6 and 5.8% exceeded 200 and 500 mg kg^{-1}, respectively. These values are higher than those reported from several countries (Table 1). The mean concentration 235 mg kg^{-1} in sauerkraut of the Czech manufacturers is significantly higher than those in household-prepared and sterilized sauerkraut (Table 3). *Pediococcus* spp. may participate considerably in TY formation, as was found during beer fermentation (Izquierdo-Pulido, Carceller-Rosa & Marinté-Font 1997).

Putrescine and tyramine concentrations (Table 2) are comparable with literature data. Their frequencies are given in Fig. 1. The significantly lowest values were observed in household-prepared sauerkraut (Table 3).

Surprisingly, histamine levels (Table 2 and Fig. 1) were usually lower than those reported in the literature (Table 1). Concentrations below the detection limit 2.1 mg kg^{-1} were observed in 43.8% of samples, while those above 10 or 20 mg kg^{-1} were only in 19.0 or 6.6% of samples, respectively.

The other three amine concentrations were often below their detection limits: 13.2, 57.9 and 88.4% for SPD, TR and SPM, respectively. The maximum SPM concentration was 33.6 mg kg^{-1} and the detectable values were mostly below 10 mg kg^{-1}. Thus, SPM data are not given in Table 2. Also occurrence of TR and SPD in sauerkraut may be considered as low, though their concentrations above 10 mg kg^{-1} were found only in 18.2 and 28.9% of samples for TR and SPD, respectively.

Characteristics of sauerkraut from six Czech manufacturers are given in Table 4 and results of analysis

Table 3
Results of analyses of variance for sample groups in order TY-PUT-CAD

Group	Austrian	Household	Sterilized
Czech	0-0-0[a]	3-3-3	2-2-0
Austria		0-3-0	0-0-0
Household			0-2-1

[a] Significance level: 3,$p<0.01$; 2, $p<0.05$; 1,$p<0.1$; 0, not significant.

Table 5
Results of analyses of variance for Czech manufactures in order TY-PUT-CAD

Manufacturer	B	C	D	E	F
A	3-0-0[a]	2-0-0	2-1-0	2-0-0	0-0-0
B		0-1-0	0-0-0	0-0-0	0-0-0
C			0-2-0	0-0-0	0-0-0
D				0-0-0	0-0-0
E					0-0-0

[a] Significance level: 3, $p<0.01$; 2, $p<0.05$; 1, $p<0.1$; 0, not significant.

Table 4
Statistical characteristics of sauerkraut samples from six Czech manufacturers

Parameter		Manufacturer A $n=11$		B $n=8$		C $n=6$		D $n=6$		E $n=6$		F $n=5$	
		$\bar{x}$	STD	$\bar{x}$	STD	$\bar{x}$	STD	$\bar{x}$	STD	$\bar{x}$	STD	$\bar{x}$	STD
Amines	(mg kg^{-1})												
HI		16.1	10.8	6.1	2.6	7.0	7.1	7.5	9.1	7.1	7.9	3.0	3.0
TY		436	230	138	123	217	139	156	139	177	107	361	343
PUT		218	96.3	166	68.5	235	66.9	134	72.4	195	155	146	114
CAD		80.8	50.0	43.4	26.1	43.2	48.6	45.1	28.1	63.1	48.4	39.6	42.3
TR		2.5	3.7	7.3	13.6	9.2	15.0	2.2	2.6	3.0	6.8	1.5	2.7
SPD		11.8	5.2	8.8	4.5	4.4	2.6	4.6	3.1	6.5	3.9	10.5	3.6
pH		3.53	0.07	3.56	0.11	3.50	0.10	3.68	0.15	3.46	0.08	3.48	0.10
Total acidity	(mg NaOH 100 g^{-1})	549	167	494	60	436	49	348	148	462	60	555	121
Lactic acid	(% w/w)	1.51	0.35	1.16	0.11	1.29	0.16	0.98	0.42	1.24	0.14	1.41	0.47
Acetic acid	(% w/w)	0.35	0.11	0.30	0.01	0.26	0.05	0.27	0.16	0.23	0.06	0.34	0.13
Alpha-amino groups	(mg 100 g^{-1})	43.2	10.1	53.8	21.2	33.0	10.5	35.5	12.5	35.3	11.3	41.0	13.9
Ammonia	(mg 100 g^{-1})	17.9	5.9	21.1	7.6	23.3	7.9	20.7	9.0	28.0	9.9	24.2	10.2
Ethanol	(% w/w)	0.74	0.46	0.29	0.07	0.17	0.03	0.23	0.09	0.22	0.07	0.28	0.10

of variance in Table 5. The sample with the extreme histamine level was excluded here (manufacturer C). Significant differences were found especially for TY between manufacturer A and the other ones. Samples from that producer also showed the highest level of ethanol but no significant correlation was found between these parameters.

No significant differences were observed between the individual BAs concentrations in household-prepared sauerkraut sampled from October to December or from March to May.

There exist the recommendations of Künsch et al. (1989) for preparation of sauerkraut with lowered BAs content: the initial fermentation should run at temperatures 15–20°C and bacterial activity can be suppressed by pasteurization as soon as total acidity reaches the value 9–10 g (as lactic acid) per kg or at pH value 4.0–3.8.

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