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Changes in biogenic amine concentrations during sauerkraut storage

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Abstract

Sauerkrauts were prepared in four laboratory experiments from three white cabbage varieties by spontaneous fermentation at 15°C for 14 days and then stored at 5–6°C and sampled after 2, 4, 6 and 12 months. Seven biogenic amines were extracted with perchloric acid and determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Sauerkraut quality parameters such as pH value, total acidity and lactic acid, acetic acid, ethanol and ammonia concentrations were also determined. Histamine, tryptamine and spermine were virtually below detection limits. Tyramine was present at the highest levels, up to hundreds mg kg^{-1}, followed by putrescine and cadaverine. Spermidine concentrations were below 14 mg kg^{-1}. Tyramine concentrations increased significantly ($P < 0.01$) with storage time. Significant regression ($P < 0.01$) was observed between the sum of the latter four amine concentrations and ammonia concentrations. No differences were observed at the significance level $P < 0.01$, between amine concentrations in sauerkrauts and corresponding sauerkraut juices.

1. Introduction

Numerous foods contain biogenic amines (BAs), a group of biologically-active natural compounds. They are formed mainly by microbial decarboxylation of amino acids. Thus, from histidine, tyrosine and tryptophan, the monoamines histamine (HI), tyramine (TY) and tryptamine (TR), respectively, are provided and, similarly, the diamines putrescine (PUT) and cadaverine (CAD) are formed from ornithine and lysine, respectively. Putrescine is a precursor for formation of the polyamines spermidine (SPD) and spermine (SPM).

An excessive oral intake of the BAs, especially of HI and TY, causes psychoactive and vasoactive effects. Lower 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, detoxification efficiency varies considerably among individuals and may be suppressed by several factors, especially by intake of some MAO inhibitors (e.g. some antidepressives or alcohol). Intake of limited amounts of polyamines, SPD, SPM and PUT in foods, may be desirable under some physiological conditions (Bardócz, 1993).

A lot of information on formation and occurrence of the BAs in foods may be found in recent reviews (Beutling, 1996; Davídek & Davídek, 1995; Shalaby, 1996; Silla Santos, 1996).

Among foods whose contents of the BAs should be taken into consideration are sauerkraut, prepared from shredded white cabbage by lactic fermentation and popular in many European countries. Data from the review of Buckenhüskes, Sabatke and Gierschner (1992) and results of our survey of 121 sauerkraut samples (Kalač, Špička, Křížek, Steidlová & Pelikánová, 1999) show high levels of TY and PUT.

The objective of the present work was to study changes in BA concentrations during long-term sauerkraut storage because such information is lacking and sauerkraut produced commonly from September to November is consumed throughout the year.

2. Materials and methods

2.1. Sauerkraut preparation

Three white cabbage varieties of different times of ripening were used. Shredded materials were taken from a sauerkraut manufacturer and laboratory experiments

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Table 1
Characteristics of shredded cabbage used in the experiments

Experiment no.	Date	Variety	Cabbage composition (% fresh matter)				
			Dry matter	Crude protein	Glucose	Fructose	Saccharose
1	28 August	Glorie (early)	7.75	0.85	2.14	1.89	0.19
2	18 September	Glorie	8.15	0.91	2.10	1.77	0.33
3	25 September	Dobrovodské (semi-late)	7.0	0.80	1.78	1.66	0.18
4	3 November	Polar (late)	9.55	0.72	2.66	2.27	0.75

were started within 3 h. Characteristics of the used cabbages are given in Table 1. The material for experiment 4 was prepared from cabbage exposed to morning frosts (−3 to −7°C) for 2 weeks prior to harvest.

Table 2
Changes in biogenic amine concentrations and in sauerkraut quality parameters in experiment 1

Parameter	Storage time (months)			
	2	4	6	12
Amines (mg kg^{-1})				
Histamine	ND[a]	ND	ND	ND
Tyramine	107	325	338	570
Putrescine	116	150	143	148
Cadaverine	28.2	31.1	24.7	23.6
Tryptamine	ND	ND	ND	ND
Spermidine	ND	13.4	13.0	12.5
Spermine	ND	ND	ND	ND
pH	3.60	3.60	3.55	3.65
Total acidity (mg NaOH 100 g^{-1})	570	590	630	630
Lactic acid (% w/w)	1.35	1.59	1.54	1.50
Acetic acid (% w/w)	0.34	0.46	0.43	0.44
Ethanol (% w/w)	0.45	0.58	0.53	0.85
Ammonia (mg 100 g^{-1})	18	27	36	36

[a] ND, not detected.

Table 3
Changes in biogenic amine concentrations and in sauerkraut quality parameters in experiment 2

Parameter	Storage time (months)			
	2	4	6	12
Amines (mg kg^{-1})				
Histamine	ND	ND	ND	ND
Tyramine	ND	85.8	162	274
Putrescine	ND	89.8	106	131
Cadaverine	1.5	5.5	12.4	24.3
Tryptamine	ND	ND	ND	ND
Spermidine	ND	8.4	6.6	8.8
Spermine	ND	ND	ND	5.3
pH	3.60	3.65	3.60	3.60
Total acidity (mg NaOH 100 g^{-1})	565	570	590	625
Lactic acid (% w/w)	1.65	1.68	1.65	1.55
Acetic acid (% w/w)	0.36	0.36	0.31	0.27
Ethanol (% w/w)	0.67	0.96	0.88	0.96
Ammonia (mg 100 g^{-1})	13	23	23	33

The material was mixed with 2% (w/w) of table salt and 660 g of mixture was filled into jars of volume 720 cm^3. The shredded cabbage was fully immersed in the released juice. The jars were closed with Omnia caps 30 min after being filled. These laboratory silos allow the escape of gases or froth produced during the initial fermentation and later are hermetic. The silos were stored in an incubator at 15°C for 14 days and then were stored in a refrigerator at 5–6°C. These conditions may be considered as optimal for good quality sauerkraut preparation. The extensive fermentation with gas formation and release of juices from the jars started within the initial 2 days and decreased on days 5 or 6. The initial cabbage pH values of about 6.0 decreased to 3.7–3.9 after 4 days and to about 3.5 after 2 weeks.

2.2. Sampling

Sauerkrauts from two jars were sampled after 2, 4, 6 and 12 months of storage in each experiment and analysed in duplicate. Sporadically, jars suspected of air access were excluded.

Table 4
Changes in biogenic amine concentrations and in sauerkraut quality parameters in experiment 3

Parameter	Storage time (months)			
	2	4	6	12
Amines (mg kg^{-1})				
Histamine	ND	ND	ND	ND
Tyramine	ND	104	175	268
Putrescine	9.8	208	233	216
Cadaverine	9.9	15.7	18.6	13.4
Tryptamine	ND	ND	ND	3.0
Spermidine	7.6	12.2	12.7	10.4
Spermine	ND	ND	ND	ND
pH	3.55	3.60	3.55	3.50
Total acidity (mg NaOH 100 g^{-1})	520	565	645	580
Lactic acid (% w/w)	1.36	1.45	1.39	1.37
Acetic acid (% w/w)	0.25	0.35	0.39	0.23
Ethanol (% w/w)	0.45	0.52	0.51	0.62
Ammonia (mg 100 g^{-1})	11	21	28	25

Table 5
Changes in biogenic amine concentrations and in sauerkraut quality parameters in experiment 4

Parameter	Storage time (months)			
	2	4	6	12
Amines (mg kg^{-1})				
Histamine	ND	ND	ND	ND
Tyramine	84.5	93.8	93.4	168
Putrescine	43.9	41.1	43.4	63.7
Cadaverine	18.0	55.0	53.3	75.9
Tryptamine	ND	ND	ND	ND
Spermidine	10.8	9.4	6.0	8.3
Spermine	ND	ND	ND	8.6
pH	3.55	3.50	3.55	3.50
Total acidity (mg NaOH 100 g^{-1})	610	695	655	615
Lactic acid (% w/w)	1.60	1.58	1.40	1.52
Acetic acid (% w/w)	0.42	0.41	0.34	0.36
Ethanol (% w/w)	0.83	0.78	0.87	1.02
Ammonia (mg 100 g^{-1})	6	9	14	15

2.3. Analytical methods

Dry matter content of shredded cabbage was determined by oven-drying to a constant weight; crude protein content was determined by the Kjeldahl method. The main fermentable sugars were extracted in boiling ethanol, evaporated to dryness under vacuum, derivatized to dimethylsilylethers and determined by a gas chromatographic method as described by Špička (1995).

Seven observed BAs were extracted from sauerkraut samples with 0.6 M $HClO_4$ and determined as *N*-benzamides by a method of micellar electrokinetic capillary chromatography, described in detail by Křížek and Pelikánová (1998), using a Spectraphoresis 2000 (Thermo Separation Products, Fremont, CA, USA). 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 by six parallel analyses

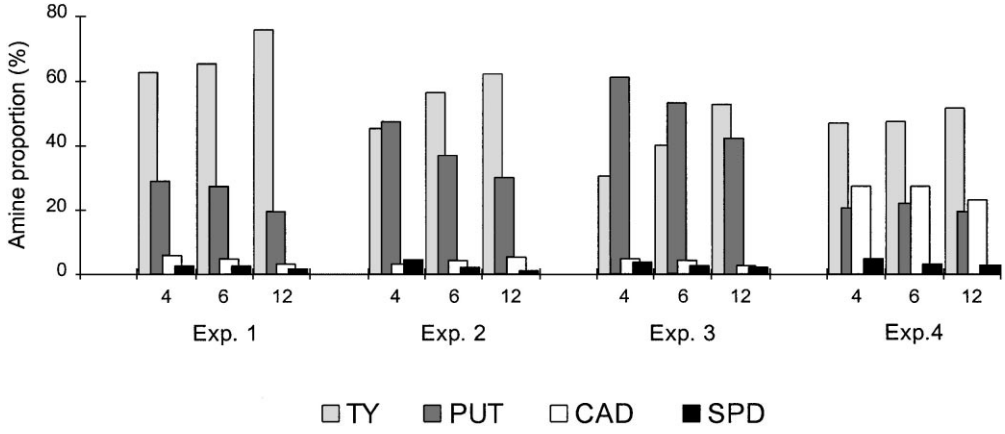

Fig. 1. Proportions of four amines in sauerkrauts after 4, 6 and 12 months storage.

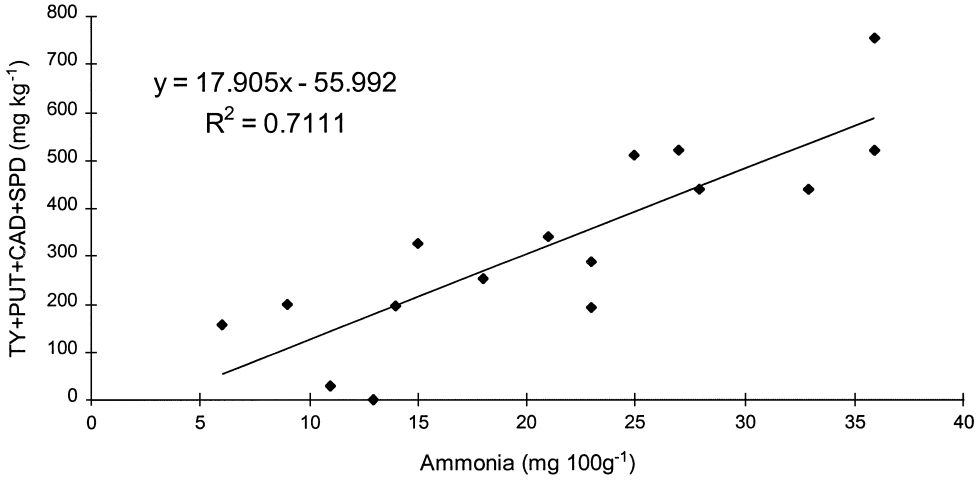

Fig. 2. Regression analysis between sum of four amine concentrations and ammonia concentrations.

of a sauerkraut sample. 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.

Methods of determination of sauerkraut quality criteria were described in our previous paper (Kalač et al., 1999). Repeatability of the analytical procedures was tested similarly to the BAs. Relative standard deviations were 0.12% at mean pH value 3.52, and 0.9, 2.3, 3.5, 5.6 and 3.0% at mean concentrations 632 mg NaOH 100 g^{-1}, 11.8 mg 100 g^{-1}, 0.79, 1.44 and 0.35 for total acidity, ammonia, ethanol, lactic acid and acetic acid, respectively.

2.4. Statistical methods

Statistical data were obtained by analysis of variance (ANOVA) and *t*-tests using Microsoft Excel 5.0.

3. Results and discussion

Changes in the BA concentrations and in sauerkraut quality parameters are given in Tables 2–5. HI was not detected, TR only in experiment 3 after 12 months storage and SPM in two experiments after 12 months

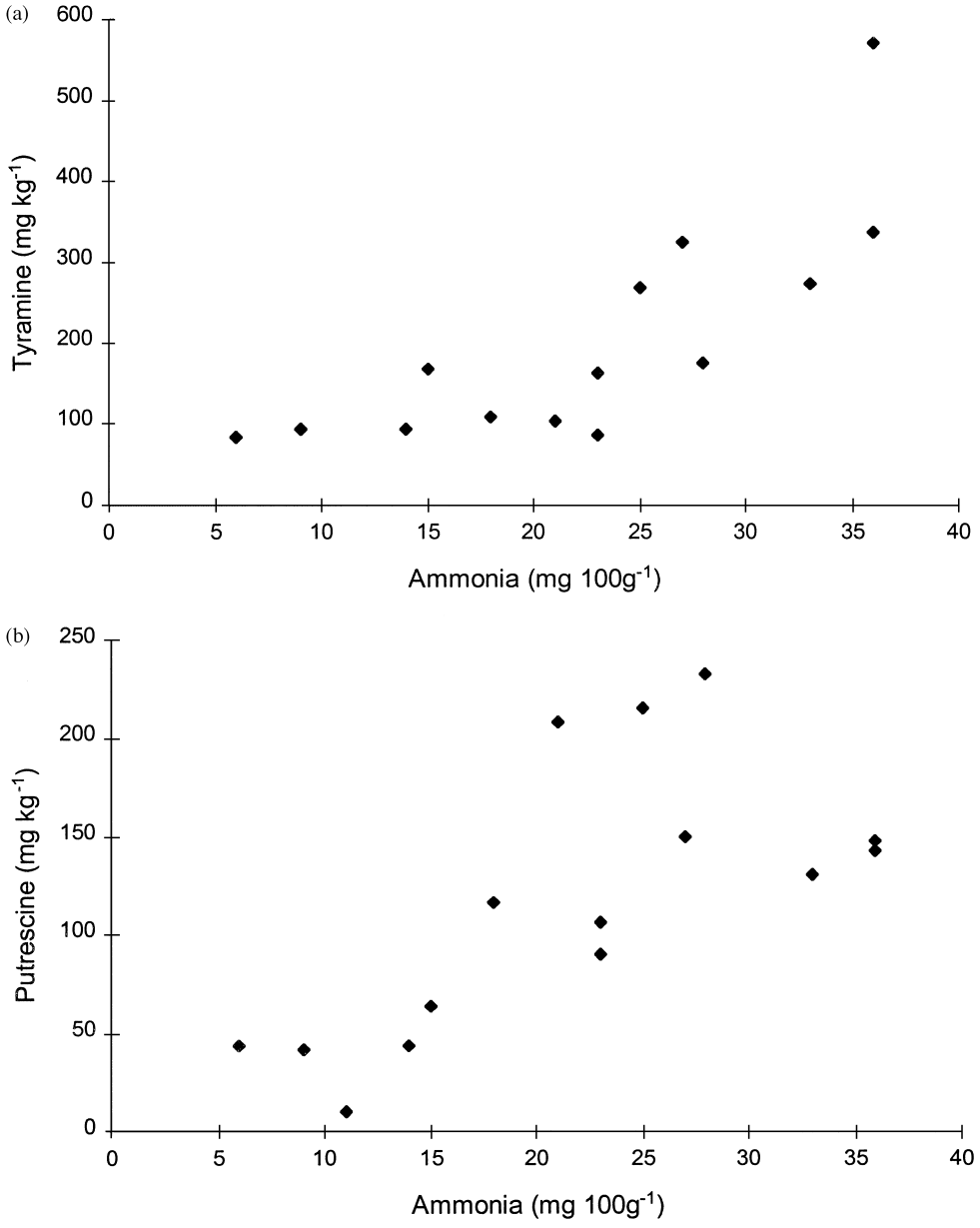

Fig. 3. Relationship between amine and ammonia concentrations.

Table 6
Amine concentrations (mg kg^{-1}) in sauerkraut and corresponding juice after 6 months storage[a]

No		Tyramine	Putrescine	Cadaverine	Spermidine	Spermine
1	Sauerkraut	310	144	26.3	13.8	ND
	Juice	346	129	30.0	14.6	ND
2	Sauerkraut	162	106	12.4	6.6	ND
	Juice	146	109	16.5	7.1	ND
3	Sauerkraut	175	260	18.6	12.7	2.1
	Juice	213	240	21.6	11.7	1.7
4	Sauerkraut	131	63.8	59.6	6.6	3.2
	Juice	387	32.1	53.4	2.4	3.1
5	Sauerkraut	44.1	21.9	38.6	8.2	17.2
	Juice	137	44.9	57.0	22.2	16.2
6	Sauerkraut	77.3	35.2	51.0	9.7	2.6
	Juice	151	61.4	52.2	15.4	2.2

[a] Histamine and tryptamine concentrations were below detection limits.

storage only. Similarly low levels of these amines were observed in our previous sauerkraut survey (Kalač et al., 1999). Thus, these three BAs are not taken into further consideration.

However, absence of detectable HI concentrations is surprising though Mayer, Pause and Vetsch (1974) reported its increase from the third week to a level of 160 mg kg^{-1} after 10 weeks simultaneously with *Pediococcus cerevisiae* appearance and Künsch, Schärer and Temperli (1989) reported its formation from the fifth day to about 105 mg kg^{-1} on day 20. This amine was determined by a fluorimetric method after separation by gel electrophoresis.

All four experiments were statistically evaluated by two-factorial ANOVA with the individual experiments and storage time as factors. Significant differences were observed among experiments for TY, PUT and CAD and for storage time for TY only at the significance level $P < 0.01$. Putrescine concentrations after 2 months were excluded from evaluation but if included, its increase during storage would also be significant.

Changes in proportions of the four amines among the experiments and during storage may be seen in Fig. 1.

Within quality parameters, significant differences ($P < 0.01$) were observed among the experiments in lactic acid, ammonia and ethanol concentrations and for storage time in ammonia concentrations only.

There was a significant statistical linear relationship between total concentrations of four BAs (TY, PUT, CAD and SPD) and ammonia concentrations (Fig. 2). However, no linear correlation was observed between ammonia concentrations and concentrations of the individual amines. As may be seen from Fig. 3, relationships for TY and PUT seems to be exponential and sigmoid, respectively. Similar results were reported by Křížek (1993) in silages prepared from orchardgrass, red clover and oats.

No simple situation for BA concentration changes may be supposed. Their bacterial formation and decomposition by oxidation are affected by many factors. Activities of amino acid decarboxylases of different bacteria are different in optimal temperature and pH ranges as was proved by Greif, Greifová, Dvoran, Karovičová and Buchtová (1999) by cultivation of several bacteria in cabbage juice. Other factors affecting bacterial growth are oxygen, NaCl and glucose concentrations. Thus, different epiphytic microflora and spontaneous fermentation cause differences in BA levels and composition.

High tyramine levels seem to be the most problematic among the BAs in sauerkraut. Some *Micrococcus* spp. exhibit tyramine oxidase activity which is, however, low under sauerkraut conditions (Leuschner, Heidel & Hammes, 1998).

As we noticed some remarks in the literature about higher BA concentrations in sauerkraut juices than corresponding sauerkrauts, we analysed six samples of each. Results are given in Table 6. No differences were observed at a significance level $P < 0.01$.

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