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The effects of lactic acid bacteria inoculants on biogenic amines formation in sauerkraut

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Abstract

Sauerkrauts were prepared in three laboratory experiments from three white cabbage varieties by initial fermentation at 22°C for 14 days, then stored at 5–6°C and analysed after six months. Six variants were observed: a spontaneously fermented one as control and inoculated ones with commercial strains of lactic acid bacteria *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus pentosaceus*, *Enterococcus faecium* and mixed Microsil preparative, all at dose 5×10^6 CFU g^{-1} of cabbage. Seven biogenic amines were extracted with perchloric acid and determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Sauerkraut quality parameters, pH value, total acidity, lactic acid, acetic acid, ammonia, alpha-amino groups and ethanol, were also determined. Histamine, tryptamine, spermidine and spermine concentrations were usually below 10 mg kg^{-1} and sometimes below detection limits. Formation of tyramine, putrescine and cadaverine, whose total concentrations were from 450 to 780 mg kg^{-1} in control variants, was significantly ($P < 0.005$) suppressed by *L. plantarum* and Microsil. Similarly, these inoculants also significantly lowered ($P < 0.005$) formation of acetic acid, ammonia and alpha-amino groups. The effects of the other inoculants were limited.

1. Introduction

Sauerkraut prepared from shredded cabbage by lactic fermentation has been a popular and widely consumed preserved vegetable in many European countries. Data from a review of Buckenhüskes, Sabatke and Gierschner (1992) and results of our survey of 121 sauerkraut samples (Kalač, Špička, Křížek, Steidlová & Pelikánová, 1999) show occurrence of biogenic amines, mainly high levels of tyramine and putrescine, in hundreds mg kg^{-1}.

Biogenic amines (BAs) are a group of biologically active natural compounds, formed mainly by microbial decarboxylation of amino acids. Thus, the monoamines, histamine (HI), tyramine (TY) and tryptamine (TR), arise from histidine, tyrosine and tryptophan, respectively. 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).

Normal intakes of the BAs are metabolized in the human 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). Detoxification efficiency varies considerably among individuals and may be supressed by several factors, mainly by intake of some MAO inhibitors (e.g. some antidepressives or alcohol). An excessive intake of BAs in foods, especially of HI and TY, cause a scale of symptoms due to their psychoactive and vasoactive effects. Intake of the limited amounts of polyamines, SPD, SPM and PUT, may be desirable under some physiological conditions (Bardócz, 1993).

The objective of the present work is to test, in laboratory experiments, the possibility of decreasing the concentrations of the seven above-mentioned amines during sauerkraut preparation with some lactic acid bacteria inoculants, as compared with sauerkraut prepared traditionally by spontaneous fermentation.

2. Materials and methods

2.1. Sauerkraut preparation

Three white cabbage varieties, of different times of ripening, were used. Shredded materials were purchased

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from a sauerkraut manufacturer and laboratory experiments were started within 2 h. Characteristics of the used cabbages are given in Table 1.

The material was mixed with 1.5% (w/w) of table salt and 25 ml kg^{-1} of a suspension of an inoculant in dose of 5×10^6 CFU g^{-1}. As a control variant for spontaneous fermentation, there was cabbage with 1.5% of salt and 25 ml of distilled water per kg. No spices were added. Jars of volume 720 cm^3, were fully filled with 660 g of the mixture. These laboratory silos allowed the escape of gases or froth produced during some 5–7 days of initial fermentation and later, after cooling, are hermetic. The shredded cabbage was fully immersed in the released juice. The jars were closed with Omnia caps 30 min after being filled. The jars were stored in a room in the dark at 22°C for 14 days (so-called warm initial fermentation); then they were stored in a refrigerator at 5–6°C. These conditions may be considered as optimal for good quality sauerkraut preparation.

2.2. Inoculants

Five commercial lactic acid bacteria preparatives were applied. The inoculants were produced by Medipharm CZ Ltd., Hustopeče near Brno, Czech Republic for silage-making. The used homofermentative strains are registered in the Czech Collection of Microorganisms (CCM) in Brno. Pure freeze-dried cultures of *Lactobacillus plantarum* (CCM 3769), *Lactobacillus casei* (CCM 3775), *Pediococcus pentosaceus* (CCM 3770) and *Enterococcus faecium* M 74 (CCM 6226) were used in a concentration of 5×10^9 CFU g^{-1} and a mixed preparative Microsil containing *L. plantarum*, *L. casei*, *E. faecium* and *Pediococcus* spp. in a total concentration of 10×10^9 CFU g^{-1}. The preparatives were suspended in distilled water immediately prior to application.

2.3. Sampling

Sauerkrauts from three jars in each of six variants were sampled after 6 months of storage and analysed as triplicates. We excluded sporadically occurring jars suspected of air access by changes of sauerkraut colour to greyish.

Table 1
Characteristics of shredded cabbages used in the experiments during 1998

Experiment no.	Date	Variety	Dry matter (g kg^{-1})
1	29 September	Glorie (early)	87.8
2	21 October	Jaguár (late)	83.5
3	27 October	Krautkaiser (late)	86.0

2.4. Analytical methods

Dry matter content of shredded cabbages and chemical quality criteria of sauerkrauts were determined as described in our previous papers (Kalač et al., 1999; Kalač, Špička, Křížek & Pelikánová, 2000). Also, information on quantitation of the observed BAs as *N*-benzamides was obtained by micellar electrokinetic capillary chromatography, described in detail by Křížek and Pelikánová (1998).

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. Relative standard deviations were 11.2, 7.8 and 7.1% for TY, PUT and CAD, respectively. Similar information for sauerkraut quality criteria is given in our above-cited papers.

2.5. Statistical methods

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

3. Results and discussion

Sauerkrauts were not evaluated sensorially due to a limited number of trained panellists. However, from the consumers' point of view, sauerkrauts prepared with *L. plantarum* and Microsil were assessed by the laboratory staff as the superior ones.

Biogenic amine concentrations and sauerkraut quality parameters are given in Tables 2–4. No other alcohols or volatile fatty acids were detected. All values are in good agreement with our previous results (Kalač et al., 1999, 2000), and comparable with values for CAD and PUT found by Andersson (1988) in a mixture of carrot, Swedish turnip, cabbage and bell pepper inoculated with *L. plantarum* at a dose of 10^6 CFU g^{-1}, analysed after 1 month of fermentation and storage. However, TY was not detected in that work.

Concentrations of TY, PUT and CAD prevailed considerably within the detected amines. The effects of the inoculants on their formation can be seen in Fig. 1. Four amines, HI, TR, SPD and SPM, were detected mostly in concentrations below 10 mg kg^{-1} and in some samples their levels were below detection limits. Thus, their biological effects on man may be considered as limited and they are not taken into further consideration.

The experiments were statistically tested by a two-factorial ANOVA test with multiple observation, with the individual experiments and the used inoculants as factors. Results of the analyses of variance are given in Table 5. Low significance level ($P<0.005$) was used because significant differences occurred between the individual experiments. The parameters of sauerkraut

Table 2
Biogenic amine concentrations and sauerkraut quality parameters in experiment 1 after 6 months storage[a]

Parameter	Control	*L. plantarum*	*L. casei*	*P. pentosaceus*	*E. faecium*	Microsil
Amines (mg kg^{-1})						
Tyramine	212	95.7	105	116	92.7	96.9
Putrescine	446	12.5	391	286	295	82.5
Cadaverine	122	11.8	78.4	79.6	75.7	26.3
Histamine	2.7	1.4	1.6	2.3	7.0	0.8
Tryptamine	6.6	1.6	2.2	3.2	2.8	3.9
Spermidine	14.6	4.9	7.7	7.1	7.6	11.0
Spermine	ND[b]	1.6	2.0	2.3	1.0	1.1
pH	3.51	3.49	3.51	3.55	3.50	3.48
Total acidity (mg NaOH 100 g^{-1})	1010	790	895	805	920	800
Lactic acid (% w/w)	1.76	1.59	1.74	1.71	1.70	1.58
Acetic acid (% w/w)	0.48	0.22	0.38	0.33	0.44	0.33
Alpha-amino groups (mg 100 g^{-1})	54	37	60	61	51	46
Ammonia (mg 100 g^{-1})	33	18	30	27	31	21
Ethanol (% w/w)	0.47	0.98	0.67	0.92	0.55	0.20

[a] Data are mean values from triplicates.
[b] ND, all three values were below detection limit.

Table 3
Biogenic amine concentrations and sauerkraut quality parameters in experiment 2 after six months storage

Parameter	Control	*L. plantarum*	*L. casei*	*P. pentosaceus*	*E. faecium*	Microsil
Amines (mg kg^{-1})						
Tyramine	108	31.6	85.9	100	107	29.5
Putrescine	327	8.7	175	165	421	8.3
Cadaverine	59.4	7.3	36.2	16.9	80.0	3.3
Histamine	4.8	2.0	0.6	1.3	3.0	1.0
Tryptamine	4.3	13.2	8.5	8.4	5.4	28.4
Spermidine	8.6	5.8	6.9	6.0	8.4	5.7
Spermine	2.5	1.2	1.7	1.7	ND	ND
pH	3.49	3.42	3.39	3.43	3.55	3.42
Total acidity (mg NaOH 100 g^{-1})	860	725	880	790	935	790
Lactic acid (% w/w)	2.04	2.11	2.26	2.20	2.14	2.14
Acetic acid (% w/w)	0.46	0.19	0.38	0.29	0.46	0.29
Alpha-amino groups (mg 100 g^{-1})	70	42	55	56	57	39
Ammonia (mg 100 g^{-1})	31	18	31	25	28	17
Ethanol (% w/w)	0.41	0.11	0.12	1.00	0.64	0.12

Table 4
Biogenic amine concentrations and sauerkraut quality parameters in experiment 3 after 6 months storage

Parameter	Control	*L. plantarum*	*L. casei*	*P. pentosaceus*	*E. faecium*	Microsil
Amines (mg kg^{-1})						
Tyramine	84.3	52.2	122	114	93.0	70.1
Putrescine	265	4.0	315	329	418	36.2
Cadaverine	97.5	4.7	129	97.3	107	68.4
Histamine	1.0	1.1	0.6	2.2	2.7	2.4
Tryptamine	2.6	2.4	4.7	1.2	3.1	4.5
Spermidine	9.4	3.6	13.8	7.8	15.6	9.4
Spermine	ND	ND	1.8	1.5	ND	2.1
pH	3.59	3.53	3.61	3.62	3.59	3.55
Total acidity (mg NaOH 100 g^{-1})	690	610	715	665	750	660
Lactic acid (% w/w)	2.03	2.01	2.06	2.10	2.08	2.01
Acetic acid (% w/w)	0.46	0.27	0.43	0.34	0.45	0.33
Alpha-amino groups (mg 100 g^{-1})	65	47	78	71	74	51
Ammonia (mg 100 g^{-1})	34	23	38	35	37	24
Ethanol (% w/w)	0.21	0.13	0.24	0.61	0.56	0.11

Table 5
Results of analyses of variance[a]

Parameter	Control	*L. plantarum*	*L. casei*	*P. pentosaceus*	*E. faecium*	Microsil
Tyramine	a	b	a	a	a	b
Putrescine	a	c	a	a	a	b
Cadaverine	a	c	a	a	a	b
Acetic acid (% w/w)	a	d	b	c	a	c
Alpha-amino groups (mg 100 g^{-1})	a	b	a	a	a	b
Ammonia (mg 100 g^{-1})	a	c	a	b	a	c

[a] Different letters in a line mean significant differences at $P < 0.005$. The letters are given in alphabetical order with decreasing concentrations of a parameter.

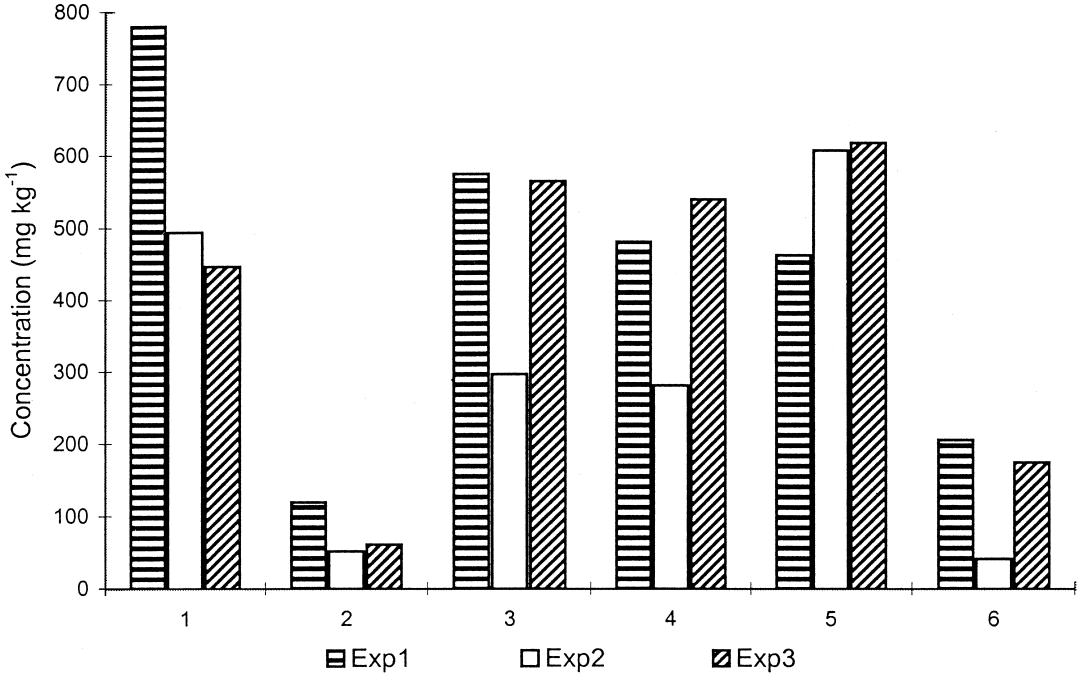

Fig. 1. Total tyramine, putrescine and cadaverine concentrations in three experiments. 1. control, spontaneous fermentation; 2. *L. plantarum*; 3. *L. casei*; 4. *P. pentosaceus*; 5. *E. faecium*; 6. Microsil.

quality with low differences (pH value, total acidity and lactic acid) showed significant differences between the experiments, while differences between the inoculants may be assessed as insignificant and they are thus not given in Table 5. Similarly, ethanol concentrations were considerably different in experiment 1 as compared to experiments 2 and 3. The lowest ethanol levels were observed in sauerkrauts inoculated with Microsil, while the highest ones were found in sauerkrauts inoculated with *P. pentosaceus* or *E. faecium*.

As can be seen from Table 5 and Fig. 1, sauerkrauts inoculated with *L. plantarum* or Microsil had significantly ($P < 0.005$) lower concentrations of TY, PUT and CAD and similarly lower levels of acetic acid, ammonia and alpha-amino groups than other variants. Thus, these two inoculants seem to be effective for improving sauerkraut quality. Research on optimization of their dosage has been in progress.

None of the tested commercial inoculants produced a statistically increased level of an amine as compared to spontaneously fermented sauerkraut. However, some strains within a species of lactic acid bacteria have the capability to produce some amines. This was reported for instance in *L. brevis* and *L. plantarum* strains associated with sausage fermentation (Straub, Kicherer, Schilder & Hammes, 1995). Each lactic acid bacteria inoculant should be tested on its amino acids decarboxylation activities prior to its use. Such a test for HI and TY production by starter cultures was suggested by Beutling (1992), and for HI, TY, PUT and CAD formation by Bover-Cid, Izquierdo-Pulido, Vidal-Carou and Holzapfel (1999).

An important factor for decrease of biogenic amine levels during sauerkraut production seems to be prevention of initial contamination by amine-producing bacteria from cabbage, shredding machines, transporters

and silos. The amine-negative inoculants should also possess competitive abilities against the contaminating microbes.

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References

Andersson, R. E. (1988). Biogenic amines in lactic acid-fermented vegetables. *Lebensmittel Wissenschaft und Technologie, 21*, 68–69.

Bardócz, S. (1993). The role of dietary polyamines. *European Journal of Clinical Nutrition, 47*, 683–690.

Beutling, D. (1992). Prüfung von Starterorganismen auf ihre Befähigung zur Bildung von Histamin und Tyramin. *Monatshefte für veterinär Medizine, 47*, 587–591.

Bover-Cid, S., Izquierdo-Pulido, M., Vidal-Carou, M. C., & Holzapfel, W. (1999). In *Proc. of the sixth workshop biologically active amines in food processing, within biogenically active amines in food.* (COST 917) (Poster 2), Prague.

Buckenhüskes, H. J., Sabatke, I., & Gierschner, K. (1992). Zur Frage des Vorkommens Biogener Amine in milchsauer fermentiertem Gemüse. Critical review. *Industrielle Obst- und Gemüsewertung, 77*, 255–262.

Kalač, P., Špička, J., Křížek, M., Steidlová, Š., & Pelikánová, T. (1999). Concentrations of seven biogenic amines in sauerkraut. *Food Chemistry, 67*, 275–280.

Kalač, P., Špička, J., Křížek, M., & Pelikánová, T. (2000). Changes in biogenic amines concentrations during sauerkraut storage. *Food Chemistry, 69*, 309–314.

Křížek, M., & Pelikánová, T. (1998). Determination of seven biogenic amines in foods by micellar electrokinetic capillary chromatography. *Journal of Chromatography A, 815*, 243–250.

Straub, B. W., Kicherer, M., Schilder, S. M., & Hammes, P. (1995). The formation of biogenic amines by fermentation organisms. *Zeitschrift für Lebensmittel Untersuchung und Forschung, 201*, 79–82.