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ORIGINAL PAPER

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Application of lactic acid bacteria starter cultures for decreasing the biogenic amine levels in sauerkraut

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Abstract Sauerkrauts from shredded white cabbage of six varieties were prepared in six laboratory experiments by initial fermentation at 22 °C for 14 days, then stored at 5–6 °C and analysed after 6 months. Seven biogenic amines were extracted with perchloric acid and determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Eight common sauerkraut quality parameters were also determined. In three experiments, a commercial strain of *Lactobacillus plantarum* and a mixed preparation of Microsil containing *L. plantarum, Lactobacillus casei, Enterococcus faecium* and *Pediococcus pentosaceus*, were applied at doses of 5×10^4, 1×10^5 and 5×10^5 CFU/g of cabbage. In three further experiments, *L. plantarum,* Microsil and a commercial strain of *Lactobacillus buchneri* were applied at doses of 5×10^5 and 5×10^6 CFU/g. Spontaneously fermented sauerkrauts were prepared as the control variants in all experiments. *L. plantarum* at doses of at least 5×10^5 CFU/g significantly ($P<0.05$) suppressed formation of putrescine, tyramine and cadaverine, amines occurring at the highest levels. Spermidine contents varied between 10 and 30 mg/kg and were not affected by the starter cultures. Levels of tryptamine, spermine and histamine were very low, often below the detection limits. For practical application, a dose of at least 5×10^6 CFU/g of the tested *L. plantarum* strain seems to be likely. The tested strains of *L. buchneri* and *E. faecium* showed tyrosine decarboxylase activity in an in vitro test.

Keywords Biogenic amines · Sauerkraut · Starter cultures · *Lactobacillus plantarum* · *Lactobacillus buchneri*

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Introduction

Sauerkraut has been a widely consumed preserved vegetable in many European countries. It has usually been prepared by spontaneous lactic fermentation of shredded cabbage, both by manufacturers and in households. Our survey of 121 sauerkraut samples [1] showed occurrence of biogenic amines at concentrations in the hundreds of mg/kg. Tyramine and putrescine were found at the highest levels.

Biogenic amines (BAs) are a group of biologically active natural compounds, arising mainly from bacterial decarboxylation of amino acids. The monoamines histamine (HI), tyramine (TY) and tryptamine (TR) and the diamines putrescine (PUT) and cadaverine (CAD) are formed from histidine, tyrosine, tryptophan, ornithine and lysine, respectively. Putrescine is a precursor of the polyamines spermidine (SPD) and spermine (SPM) [2].

Usual intakes of dietary BAs are metabolised by a fairly efficient detoxification system based on intestinal and hepatic amine oxidases. Detoxification efficiency varies considerably among individuals and can be suppressed by several factors, mainly by the intake of the amine oxidases inhibitors in some drugs. An excessive intake of BAs in foods, especially of HI and TY, cause a scale of symptoms due to their psychoactive and/or vasoactive effects. For instance, TY and HI participate in dietary migraines and food intolerances [3]. Rapidly increasing current knowledge on the polyamines PUT, SPD and SPM indicates their role in tumour growth [3].

Thus, levels of BAs in foods should be decreased. As we observed in our previous work [4], application of selected commercial lactic acid bacteria starter cultures (inoculants) at a dose of 5×10^6 CFU/g of cabbage significantly decreased the BA levels in sauerkraut.

The objective of the present work is to optimise, in laboratory experiments, doses of the promising previously tested strain of *Lactobacillus plantarum* and of the mixed commercial Microsil preparation in order to decrease levels of the above-mentioned amines, as compared with sauerkraut prepared traditionally by spontane-

ous fermentation. Moreover, a commercial strain of *Lactobacillus buchneri* was tested as this heterofermentative bacterium has been known to improve aerobic stability of silages. All the starter cultures were also tested for their ability to produce BAs under in vitro conditions.

Materials and methods

Sauerkraut preparation

Six white cabbage varieties were used. Shredded materials were purchased 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 of a starter culture suspension. The same volume of distilled water was used instead of the suspension in a control variant for spontaneous fermentation. Jars of volume 720 mL were fully filled with 660 g of the mixture, which was fully immersed in the released juice. The jars were closed with Omnia caps and stored in the dark at 22 °C for 14 days and then they were stored in a refrigerator at 5–6 °C. These conditions can be considered as optimal for good quality sauerkraut preparation.

Starter cultures

Three commercial lactic acid bacteria preparations were applied. The inoculants were produced by Medipharm CZ Ltd., Hustopeče near Brno, Czech Republic, for silage making. The used strains are registered in the Czech Collection of Microorganisms (CCM) in Brno. Pure freeze-dried cultures of *Lactobacillus plantarum* (CCM 3769), *Lactobacillus buchneri* (CCM 1819) and the mixed preparation Microsil containing *L. plantarum* (CCM 3769), *Lactobacillus casei* (CCM 3775), *Enterococcus faecium* (CCM 6226) and *Pediococcus pentosaceus* (CCM 3770) were applied in doses from 5×10^4 to 5×10^6 CFU/g of shredded cabbage. The preparations were suspended in distilled water immediately prior to application.

Sampling

Sauerkrauts from three jars in each of seven variants (including the control) were sampled after 6 months of storage and analysed as triplicates. The occasional jars that were not closed hermetically were excluded.

Amino acid decarboxylase activity of the starter cultures

The pure cultures of each microorganism used as the inoculant were tested for BA formation following the procedure described by Bover-Cid and Holzapfel [5]. First, cultures were subcultured five times in Man Rogosa and Sharpe (MRS) broth containing the precursor amino acids and pyridoxal phosphate. Afterwards, they were inoculated in tubes with decarboxylase medium and incubated at 30 °C for 4 days. Finally, BAs in the fermenting broth were determined by high performance liquid chromatography.

Analytical methods

Dry matter content of shredded cabbages and chemical quality criteria of sauerkrauts were determined as described in the previous papers [1, 4]. Analytical procedure for determination of the observed BAs as *N*-benzamides, using micellar electrokinetic capillary chromatography, has been described in detail by Křížek and Pelikánová [6].

Table 1 Characteristics of shredded cabbages used in the experiments

Experiment no.	Date	Variety	Dry matter (g/kg)
1	9 September 1999	Manama (early)	69.3
2	15 September 1999	Krautkaiser (late)	77.0
3	13 October 1999	Jaguár (late)	84.3
4	4 October 2000	Selma (late)	65.0
5	18 October 2000	Oklahoma (late)	72.6
6	1 November 2000	Transam (late)	89.7

The detection limits were 1.0, 1.3, 1.4, 1.4, 2.1, 2.1 and 3.5 mg/kg 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 quality criteria was given in the above-cited papers [1, 4].

Statistical methods

Statistical data were obtained by *t*-tests with unequal distribution at significance level $P<0.05$ using Microsoft Excel. Contents of biogenic amines below the detection limits were used for the calculations as halves of the limits (e.g. 0.7 mg/kg for CAD in samples below the detection limit of 1.4 mg/kg).

Results and discussion

The in vitro study of amino acid decarboxylase activity of the inoculants used in this study showed that only the commercial *Lactobacillus buchneri* and the *Enterococcus faecium* from the Microsil preparation were amine producers. Particularly, they decarboxylated tyrosine producing similar TY amounts (1700 mg/mL of broth). Indeed, these species have often been described as strong TY producers [5].

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

Tables 2, 3 and 4 give the biogenic amine contents and sauerkraut quality parameters in experiments 1–3, using doses of *L. plantarum* and Microsil lower than in our initial experiments [4].. No other alcohols (C_3 or C_4) or volatile fatty acids (C_3–C_6) were detected at the detection limits of 0.01–0.02% (w/w). Levels of all parameters were in good agreement with our previous results.

PUT and TY were determined at the highest concentrations, CAD and SPD at medium or low levels, and HI, TR and SPM were found irregularly and at very low concentrations. Thus, levels of the latter amines were not analysed statistically. Statistical analyses showed no significant differences among the experimental variants in TY and PUT contents, due to wide variations within triplicates. Similarly, SPD levels were relatively stable. This amine probably originates from the used cabbage. Several significant differences were observed in lactic acid, alpha-amino groups and ammonia contents. How-

Table 2 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Manama in experiment [1] with different inoculant doses (CFU/g) after 6 months storage[1]

Parameter	Control	*Lactobacillus plantarum*			Microsil		
		5×10^4	1×10^5	5×10^5	5×10^4	1×10^5	5×10^5
Amines (mg/kg)							
Tyramine	171	128	125	120	129	137	123
Putrescine	302	333	297	395	396	253	310
Cadaverine	19.0[a,2]	12.5[a,b]	3.3[c]	1.6[c]	8.8[b,c]	2.8[c]	3.2[c]
Histamine	ND[3]	ND	ND	ND	ND	ND	ND
Tryptamine	ND	ND	ND	3.2	2.5	ND	1.7
Spermidine	16.9[a]	11.6[a]	11.9[a]	10.5[b]	13.7[a]	10.8[a]	13.8[a]
Spermine	ND	ND	ND	ND	ND	ND	ND
Quality parameters							
pH	3.64	3.56	3.54	3.57	3.57	3.58	3.57
Total acidity (mg NaOH/100 g)	750	715	725	720	715	725	725
Lactic acid (% w/w)	1.78[a]	1.78[a]	1.57[b]	1.51[b]	1.51[b]	1.36[b]	1.41[b]
Acetic acid (% w/w)	0.36	0.37	0.32	0.32	0.32	0.31	0.31
Alpha-amino groups (mg/100 g)	85[a]	67[b]	72[a,b]	63[b]	67[b]	67[b]	59[b]
Ammonia (mg/100 g)	36[a]	28[b]	24[c]	23[c]	25[b]	25[b]	18[c]
Methanol (% w/w)	0.07	0.10	0.08	0.06	0.06	0.09	0.06
Ethanol (% w/w)	0.38	0.32	0.29	0.30	0.31	0.30	0.29

[1] Data are mean values from triplicates
[2] Different letters in a line mean significant differences at $P<0.05$. The letters are given in alphabetical order with decreasing levels of a parameter
[3] ND, all three values were below detection limit

Table 3 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Krautkaiser in experiment 2 with different inoculant doses (CFU/g) after 6 months storage

Parameter	Control	*Lactobacillus plantarum*			Microsil		
		5×10^4	1×10^5	5×10^5	5×10^4	1×10^5	5×10^5
Amines (mg/kg)							
Tyramine	166	183	157	162	180	144	180
Putrescine	214	266	173	191	190	226	267
Cadaverine	42.9[a]	25.5[a,b]	5.8[b]	12.9[a,b]	46.0[a]	15.0[a,b]	13.2[a,b]
Histamine	ND	2.7	ND	ND	3.6	ND	ND
Tryptamine	2.4	4.6	1.8	1.4	1.8	4.7	3.2
Spermidine	11.9	6.5	13.4	16.1	13.4	17.0	16.6
Spermine	ND	ND	ND	ND	ND	1.5	1.6
Quality parameters							
pH	3.55	3.51	3.49	3.51	3.52	3.50	3.51
Total acidity (mg NaOH/100 g)	675	670	715	730	715	705	715
Lactic acid (% w/w)	1.32[b]	1.35[a]	1.48[a]	1.43[a]	1.39[a]	1.35[a,b]	1.46[a]
Acetic acid (% w/w)	0.30	0.31	0.33	0.31	0.31	0.29	0.33
Alpha-amino groups (mg/100 g)	57[a]	51[a,b]	46[b]	53[a,b]	52[a,b]	51[a,b]	52[a,b]
Ammonia (mg/100 g)	19	16	15	15	11	15	17
Methanol (% w/w)	0.06	0.08	0.05	0.03	0.04	0.04	0.09
Ethanol (% w/w)	0.33	0.36	0.35	0.41	0.35	0.34	0.39

ever, these results are not in a simple relation to the BA levels.

Thus, the applied doses of the both inoculants seem to be too low to decrease the BA levels, even when a component of the Microsil preparative (the *Enterococcus faecium* strain) was shown to produce TY in vitro. The expression of amino acid decarboxylase activity of microorganisms depends on multiple factors, including environmental conditions [5]. Therefore, the conditions occurring during real sauerkraut fermentation were probably much less favourable than those of the in vitro study. Bover-Cid et al. [7] reported a similar observation during sausage fermentation, since TY-producing *Lactobacillus curvatus* strain was able to slightly reduce the accumulation of this amine in comparison to the spontaneously fermented sausage (control process).

Our findings are different from those of Halász et al. [8], who tested two strains of *L. plantarum* and one strain of *Lactobacillus curvatus* and recommended starter culture doses from 5×10^4 to 2×10^5 CFU/g of cabbage. The lower dose was recommended for *L. curvatus* to avoid increased TY levels, due to its tyrosine decarboxylating activity. However, as compared with our experiments, higher doses of table salt (2 and 5% w/w) were applied and the initial fermentation was carried out at 11 °C or 30 °C.

In experiments 4–6 we, therefore, tested *L. plantarum*, Microsil and *L. buchneri* at doses of 5×10^5 and 5×10^6 CFU/g of cabbage. The results are given in Tables 5, 6 and 7.

L. buchneri, the obligate heterofermentative bacterium, was observed to metabolise lactic acid to acetic acid

Table 4 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Jaguár in experiment 3 with different inoculant doses (CFU/g) after 6 months storage

Parameter	Control	*Lactobacillus plantarum*			Microsil		
		5×10^4	1×10^5	5×10^5	5×10^4	1×10^5	5×10^5
Amines (mg/kg)							
Tyramine	206	204	209	132	148	150	102
Putrescine	368	453	475	249	448	558	322
Cadaverine	18.6	17.8	16.9	17.7	12.0	13.9	15.6
Histamine	ND	ND	ND	ND	ND	ND	ND
Tryptamine	3.7	4.0	4.5	2.0	8.5	6.7	ND
Spermidine	10.1	11.6	13.5	10.7	11.6	14.1	9.7
Spermine	ND	ND	ND	ND	3.2	ND	ND
Quality parameters							
pH	3.65	3.67	3.66	3.63	3.62	3.62	3.63
Total acidity (mg NaOH/100 g)	710	780	755	795	765	765	760
Lactic acid (% w/w)	1.02	1.08	1.03	1.11	1.06	1.08	1.06
Acetic acid (% w/w)	0.26	0.28	0.27	0.28	0.28	0.26	0.26
Alpha-amino groups (mg/100 g)	67^{b}	77^{a}	75^{a}	80^{a}	74^{a}	$71^{a,b}$	75^{a}
Ammonia (mg/100 g)	$25^{a,b}$	$27^{a,b}$	27^{a}	24^{b}	20^{c}	18^{c}	21^{c}
Methanol (% w/w)	0.06	0.05	0.04	0.05	0.04	0.03	0.03
Ethanol (% w/w)	0.19^{a}	0.21^{a}	0.19^{a}	0.19^{a}	0.14^{a}	0.11^{b}	0.18^{a}

Table 5 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Selma in experiment 4 with different inoculant doses (CFU/g) after 6 months storage

Parameter	Control	*Lactobacillus plantarum*		Microsil		*Lactobacillus buchneri*	
		5×10^5	5×10^6	5×10^5	5×10^6	5×10^5	5×10^6
Amines (mg/kg)							
Tyramine	60.5[1]	39.4^{a}	23.5^{c}	46.2^{a}	$40.8^{a,b}$	$40.2^{a,b}$	37.7^{b}
Putrescine	162^{b}	97.1^{b}	24.7^{c}	158^{b}	201^{a}	235^{a}	86.3^{b}
Cadaverine	24.0^{a}	2.5^{b}	1.8^{b}	6.5^{b}	5.6^{b}	ND^{b}	ND^{b}
Histamine	ND	ND	ND	ND	ND	ND	ND
Tryptamine	4.4	3.1	3.1	3.6	2.5	ND	2.2
Spermidine	28.0	25.4	19.4	24.3	22.1	21.5	13.5
Spermine	ND	ND	2.0	ND	ND	ND	1.4
Quality parameters							
pH	3.65^{a}	3.56^{b}	$3.49^{b,c}$	3.45^{d}	3.44^{d}	3.51^{c}	3.51^{c}
Total acidity (mg NaOH/100 g)	690^{a}	655^{a}	615^{b}	680^{a}	675^{a}	$655^{a,b}$	625^{b}
Lactic acid (% w/w)	1.46	1.43	1.42	1.51	1.41	1.47	1.49
Acetic acid (% w/w)	0.37^{a}	$0.31^{b,c}$	$0.28^{b,c}$	$0.34^{a,b}$	$0.32^{a,b}$	$0.31^{a,b}$	0.26^{c}
Alpha-amino groups (mg/100 g)	62^{a}	47^{b}	41^{b}	63^{a}	57^{a}	59^{a}	63^{a}
Methanol (% w/w)	0.03	0.03	0.03	0.03	0.03	0.03	0.02
Ethanol (% w/w)	0.18^{a}	$0.14^{b,c}$	0.12^{c}	$0.16^{a,b}$	$0.13^{b,c}$	0.15^{b}	0.09^{c}

[1] Tyramine level in control variant was not analyzed statistically due to high variability within triplicate

and 1,2-propanediol in anaerobic cultures [9]. Undissociated acetic acid content was identified as the most important inhibitory factor of yeast growth in spontaneously fermented silages. Acid-tolerant yeasts oxidise residual fermentable carbohydrates, lactic acid and acetic acid to carbon dioxide in both silage and sauerkraut exposed to air during their handling. As this process proceeds, the pH value rises and other aerobic microorganisms start to proliferate. Sensorial properties of sauerkraut become worse. Thus, the role of *L. buchneri* as compared to homofermentative starter cultures is mainly to increase aerobic stability of sauerkraut. However, the strain of *L. buchneri* used in these experiments did not significantly change the levels of acetic acid or the ratio of lactic acid to acetic acid content as compared to the other variants.

Levels of BAs in the control variants were similar to those in experiments 1–3. HI contents were even below the detection limit and the contents of SPM and TR were very low. SPD levels were similar in all three experiments and statistical differences among the variants were observed only in experiment 5 (Table 6). Thus, only the contents of TY, PUT and CAD differed statistically in all three experiments.

The greatest efficiency for decreasing the levels of the latter three amines was observed with *L. plantarum*, which was not able to decarboxylate amino acids under in vitro conditions, mainly at a dose of 5×10^6 CFU/g. Comparable levels of PUT and CAD (TY was not determined) were reported by Andersson [10] in a mixture of carrot, Swedish turnip, cabbage and bell pepper inoculat-

Table 6 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Oklahoma in experiment 5 with different inoculant doses (CFU/g) after 6 months storage

Parameter	Control	*Lactobacillus plantarum*		Microsil		*Lactobacillus buchneri*	
		5×10^5	5×10^6	5×10^5	5×10^6	5×10^5	5×10^6
Amines (mg/kg)							
Tyramine	47.4[a]	43.5[a]	36.8[b]	38.3[a,b]	33.6[b]	37.3[a,b]	42.4[a]
Putrescine	163[a]	48.1[b]	25.0[b]	173[a]	135[a]	185[a]	171[a]
Cadaverine	36.6[a]	5.3[c]	2.4[c]	14.4[b]	5.5[c]	13.0[b]	6.5[c]
Histamine	ND	ND	ND	ND	ND	ND	ND
Tryptamine	ND	2.1	ND	ND	ND	ND	ND
Spermidine	24.6[a,b]	27.2[a]	19.5[b]	22.7[a,b]	25.8[a,b]	28.8[a]	20.7[a,b]
Spermine	1.9	ND	ND	4.9	3.4	3.8	2.7
Quality parameters							
pH	3.52	3.49	3.49	3.54	3.53	3.55	3.53
Total acidity (mg NaOH/100 g)	650[a,b]	650[a,b]	640[b]	705[a]	685[a,b]	665[a,b]	670[a,b]
Lactic acid (% w/w)	1.52	1.53	1.59	1.60	1.65	1.56	1.60
Acetic acid (% w/w)	0.40[a,b]	0.39[a,b]	0.34[b]	0.42[a]	0.41[a,b]	0.37[b]	0.37[b]
Alpha-amino groups (mg/100 g)	52[a]	44[a,b]	42[a,b]	53[a]	50[a,b]	52[a]	54[a]
Ammonia (mg/100 g)	27[c]	26[c]	24[c]	32[a]	29[b]	31[a]	31[a]
Methanol (% w/w)	0.03	0.03	0.03	0.03	0.04	0.03	0.03
Ethanol (% w/w)	0.71[a]	0.89[a]	0.84[a]	0.63[a,b]	0.62[b]	0.75[a]	0.75[a]

Table 7 Biogenic amine contents and quality parameters of sauerkraut prepared from cabbage var. Transam in experiment 6 with different inoculant doses (CFU/g) after 6 months storage

Parameter	Control	*Lactobacillus plantarum*		Microsil		*Lactobacillus buchneri*	
		5×10^5	5×10^6	5×10^5	5×10^6	5×10^5	5×10^6
Amines (mg/kg)							
Tyramine	58.1[a]	44.4[b]	33.6[b]	52.5[a,b]	42.5[a,b]	39.3[b]	40.4[b]
Putrescine	46.2[b]	46.7[b]	7.1[c]	147[a]	150[a]	207[a]	51.8[b]
Cadaverine	24.4[b]	22.2[b]	4.8[c]	57.8[a]	28.8[b]	38.1[b]	6.2[c]
Histamine	ND	ND	ND	ND	ND	ND	ND
Tryptamine	2.3	2.8	9.3	ND	ND	ND	2.5
Spermidine	27.9	22.9	18.4	20.8	20.6	20.4	15.3
Spermine	2.0	ND	ND	ND	ND	ND	ND
Quality parameters							
pH	3.51[a,b]	3.51[b,c]	3.48[b,c]	3.56[a]	3.54[a,b]	3.55[a,b]	3.53[b]
Total acidity (mg NaOH/100 g)	705[b,c]	715[c]	675[c]	755[b]	750[a,b]	780[a]	700[b]
Lactic acid (% w/w)	1.74[b]	1.72[b]	1.69[c]	1.86[a,b]	1.79[b]	1.88[a]	1.90[a]
Acetic acid (% w/w)	0.34[a,b,c]	0.35[b]	0.32[b]	0.42[a]	0.39[a]	0.38[a]	0.28[c]
Alpha-amino groups (mg/100 g)	55[d]	53[d]	53[d]	71[c]	73[b]	82[a]	74[b]
Ammonia (mg/100 g)	26[b]	27[b]	25[b]	35[a]	37[a]	40[a]	39[a]
Methanol (% w/w)	0.03	0.03	0.04	0.04	0.04	0.04	0.03
Ethanol (% w/w)	0.12[a]	0.12[a,b]	0.11[a,b]	0.16[a]	0.11[a,b]	0.12[a]	0.08[b]

ed with *L. plantarum* at a dose of 10^6 CFU/g, analysed after one month of fermentation and storage.

Inoculation with *L. buchneri* or Microsil, mainly at the higher dose, as compared with the control variants, resulted in a decrease of CAD content. TY levels were affected only to a limited extent, probably because of the tyrosine decarboxylase activity of the inoculants. Surprisingly, PUT levels were comparable or even increased (although not always significantly from the statistical analysis). That observation differs from our initial results where PUT levels were significantly lowered in sauerkrauts prepared with Microsil in all three experiments [4].

L. plantarum was also the most effective in depressing proteolysis as measured by alpha amino groups and ammonia levels. Assessing the other quality parameters, the roles of the inoculants were confused. Methanol, probably originating from pectin, was present in all experiments and variants at a stable level of about 0.03% (w/w).

In conclusion, inoculation of cabbage with the tested strain of *L. plantarum* at a dose of at least 5×10^5 CFU/g significantly decreased PUT, CAD and TY contents as compared with spontaneous fermentation. Moreover, this inoculant depressed the degree of proteolysis and resulted in a favourable sensorial evaluation of the sauerkrauts. The recommended dose of *L. plantarum* for practical use is at least 5×10^6 CFU/g due to sub-optimal conditions for fermentation and storage as compared with

those in our experiments. The dose could be decreased using other strains with higher propagation rate. Nevertheless, a relatively low pH value and a low acetic acid content are factors indicating a risk of low stability against aerobic deterioration during handling of sauerkraut and its aeration enabling yeast growth [11]. Efficiency of both the mixed preparation Microsil and the tested strain of *L. buchneri* were limited.

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References

1. Kalač P, Špička J, Křížek M, Steidlová Š, Pelikánová T (1999) Food Chem 67:275-280
2. Halász A, Baráth A, Simon-Sarkadi L, Holzapfel W. (1994) Trends Food Sci Technol 5:42–49
3. Bardócz S (1995) Trends Food Sci Technol 6:341–346
4. Kalač P, Špička J, Křížek M, Pelikánová T (2000) Food Chem 70:355-359
5. Bover-Cid S, Holzapfel WH (1999) Int J Food Microbiol 53:33–41
6. Křížek M, Pelikánová T (1998) J Chromatogr A 815:243-250
7. Bover-Cid S, Hugas M, Izquierdo-Pulido M, Vidal-Carou M C (2000) J Food Protect 63: 237–243
8. Halász A, Baráth A, Holzapfel WH (1999) Z Lebensm Unters Forsch A 208:434–438
9. Driehuis F, Oude Elferink SJWH, Spoelstra SF (1999) J Appl Microbiol 87:583–594
10. Andersson RE (1988) Lebensm Wiss Technol 21:68–69
11. Petaja E, Myllyniemi P, Petaja P, Ollilainen V, Piironen V (2000) Agric Food Sci Finland 9:37–48