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Use of inoculated lactic acid bacteria in fermenting sour cabbage

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Fermentation of sour vegetables has to date occurred through the use of lactic acid bacteria (LAB) naturally present in vegetables. The present article deals with preliminary studies on the effects of some LAB inocula (*Lactobacillus alimentarius* or *Pediococcus pentosaceus*) on fermenting sour cabbage. The effect of LAB on yeast growth, a problem in sour vegetables, was also studied through the use of dual yeast and LAB inocula.

The pH of cabbage juice decreased to levels under pH 4 during the first 10 days of fermentation, which is near the final values, pediococci decreasing the pH to the lowest values. The LAB count in inoculated cabbages increased by 0.5–2.0 log cfu (colony forming unit) /g during the first 10 days of fermentation and thereafter decreased. Pediococci formed predominant part of microbial flora almost in all experimental batches. In cabbage challenged with yeasts, yeast counts rose only when the pH was < 3.5. Yeasts appeared almost regularly also in cabbages inoculated only with LAB.

Pediococci fermented cabbage effectively decreasing the pH to lower levels than lactobacilli or natural LAB. However, too strong a decrease in pH may result in a decrease of LAB count which may subsequently lead to yeast growth. The yeast problem could not be solved with the LAB inocula used in our study.

Key words: fermented foods, sauerkraut, vegetables

Introduction

Souring is a proven method for preparing vegetables as food in many areas of the world. Acidification can be carried out through the use of organic acids or by fermentation, which is the original method for preparing sour vegetables. Natural lactic acid bacterial (LAB) flora ferment the sugars in vegetables to lactic acid and small amounts of other organic acids. Salting vegetables before fermentation and acid production by LAB aids LAB in growing over other bacteria and becoming predominant microbes of the flo-

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ra. The fermentation temperature should be 20–24°C, since higher temperatures often permit growth of undesirable microbes (ICMFS 1980).

In the first phase of natural cabbage fermentation leuconostocs are the main type of LAB and are later replaced by lactobacilli as the predominant microbes of the microbial flora (Frazier 1958, Vaughn 1985, Buckenhüskes 1997). Buckenhüskes (1993) used *Leuconostoc mesenteroides* as a starter inoculum in sour cabbage fermentation. During storage leuconostocs were overgrown by *Lactobacillus plantarum*. Also pediococci have been shown to replace leuconostocs at later stage of fermentation (ICMFS 1980, Vaughn 1985). Delclos (1992) reports that mixed starter culture composed of *Leuconostoc mesenteroides* and *Lactobacillus pentosus* gave the closest resemblance to the product obtained following a natural commercial fermentation.

Fermentation of sour vegetables has to date occurred through the use of LAB naturally present in vegetables, whereas in many foods, especially in milk and meat products inoculation of specific LAB strains has been used to improve quality and reproducibility. The present paper deals with the preliminary studies in which the effects of different artificial inocula on fermenting sour cabbage are examined. The effect of LAB on yeast growth, a problem in sour vegetables, was also studied through the use of dual yeast and LAB inocula.

Material and methods

Preparation of sour cabbage

The sour cabbage was prepared using 12 kg cabbage, 2 kg carrots, 0.7 kg onions, 0.2 kg salt and 0.1 kg garlic. Cabbage (the race: rinda) originated from the same batch. Cabbage was sliced with a cutter (Seydelman Rasant 40; Seydelman Gmbh, Germany) into strips 5 mm in width. Carrots and onions were sliced with a vegetable cutter (Hälde RG 7, Metos, Finland) into strips 5 mm in diameter, and garlic was cut with a knife into small pieces. The raw materials were mixed and placed in 15-kg plastic pot by layers, adding salt after each layer. An additional 1.5 l of water with bacterial inoculum were added after the last 2 layers. The experimental batches were closed tightly, kept at 22°C for 7 days and thereafter at 6–7°C for 5 months. The sour cabbage was long ripened type; the product was ready 3 months after preparation. The experimental batches were sampled by taking sour cabbage juice through the velvets near the bottom of plastic pot. Samples were taken immediately after preparation and after 10 and 21 days of fermentation and 2, 3 and 5 months of storage.

Lactic acid bacteria inoculated

The commercial starter *Lactobacillus alimentarius* (Flora Carn FM, Chr. Hansen A/S, Hörsholm, Denmark) as a rapid acid producer and *Pediococcus pentosaceus* strain POHK (porkkanahapankaali) as a strong acid producer were selected for use as inocula. *L. alimentarius* was isolated from a commercial preparation and *P. pentosaceus* strain POHK from sour carrot strips (Petäjä and Puolanne 1997) on APT (all-purpose agar containing Tween) agar / pH 5.6 (BBL 10916). The strain POHK was identified as belonging to the species *Pediococcus pentosaceus*. Both strains were stored in APT agar at 4°C. Both LAB strains were inoculated into the vegetable mixture as APT broth culture grown for 2 days at 30°C. APT broth culture (400 ml) was mixed with 1.5 l water, and this mixture added to the mixture of vegetable strips (15 kg). The APT broth culture count was about 8.7 log cfu (colony forming unit) /ml, and the aim count in the vegetable strip mixture was 7 log cfu/g.

Yeasts

The yeast strain used was isolated from good quality sour cabbage containing yeast as contam-

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inant. The strain was isolated on Rose-Bengal agar (Labm lab36 and X085) at 25°C and maintained on Rose-Bengal agar at 4°C. The yeast strain was identified as belonging to the species *Candida sake* and it proved facultatively fermentative. The yeast strain was inoculated into the vegetable mixture as a nutrient broth (composition: 5 g peptone, 3 g meat extract and 1 g glucose in 1 l water) culture grown for 2 days at 30°C; 10 ml nutrient broth culture was mixed with 1.5 l water which was added to the vegetable mixture. The nutrient broth culture count was 6.8 log cfu/ml; the aim count in the vegetable mixture was 3 log cfu/g.

Grouping of sour cabbages

The purpose was to examine how *L. alimentarius* and *P. pentosaceus* strain POHK ferment sour cabbage and the effect of these bacteria on the growth of yeast in sour cabbage. The following groups of cabbage were prepared according to the microbial inocula:

1 Control, no inoculation (Control in tables)
2 *L. alimentarius* (400 ml) (Lactob. in tables)
3 *L. alimentarius* (200 ml) + *P. pentosaceus* strain POHK (200 ml) (Lactob. + Ped. in tables)
4 *L. alimentarius* (200 ml) + *C. sake* yeast strain (10 ml) (Lactob. + yeast in tables)
5 *P. pentosaceus* strain POHK (400 ml) (Ped. in tables)
6 *P. pentosaceus* strain POHK (400 ml) + *C. sake* yeast strain (10 ml) (Ped. + yeast in tables)

Lactobacilli and pediococci were added as an APT broth culture, yeast as a nutrient broth culture. Three experimental series of each group of sour cabbage were prepared; the microbiological results of each are presented separately because in series III the pH of 4 cabbage groups (3–6) was much lower than the pH levels of the corresponding groups in the other series and because then the predominating bacterial group of different experimental series can be enclosed behind the counts.

The pH value and titrated acid

The pH value was measured directly from the sour cabbage juice by an Ingold 104043041 electrode (Ingold Messentechnik AG, Utdorf, Switzerland). Acid titrations were conducted with 0.1-N NaOH on the filtrates (20 ml) obtained from sour vegetable juice by filtering through filter paper (Whatman 4 Cat No 1004090, 90 mm ∅), and the results were calculated as percentages (wt/vol) of sour cabbage juice. The pH values and titrated acid were determined from the fresh cabbage juice (0 days), and from the sour juice 10 and 21 days and 2, 3 and 5 months after preparation.

Redox potential and water activity value

The redox potential of the sample cabbage juices was measured using an Inlab 501 electrode (Mettler Toledo, Utdorf, Switzerland) directly from juices 10 and 21 days and 2, 3 and 5 months after preparation. The a_w values (water activity values) were measured with a Luft -a_w measurer (Luft, G. Luft Mess und Regelteknik Gmbh, Germany) after 10 and 21 days of fermentation.

Sugars

The levels of sucrose, D-glucose and D-fructose were determined using a UV method based on the measurement of the stoichiometrically formed NADPH (cat. no. 716260, Boehringer-Mannheim, Germany). The sample solution was clarified using Carrez reagent precipitation according to the instruction leaflet. UV spectrophotometric measurements were performed with a Lambda Bio UV/VIS spectrometer (Perkin-Elmer, USA). A recovery value of 94.1% (n = 3) for D-glucose was obtained.

Organic acids

Acetic acid (99.8%; Riedel de Haen, nr. 33209, Germany) and DL-lactic acid (~90%; Fluka, nr. 69785, Germany) were used as calibrants. The contents of lactic acid and acetic acid were determined using Waters liquid chromatography equipment including a chromatography pump, autosampler, air-bath column temperature module and UV detector. Chromatographic data were collected and processed with a Millennium Chromatography Manager software package (Waters, USA).

The organic acid fraction was purified with disposable strong anion-exchange (SAX) solid-phase extraction (SPE) cartridges prior to liquid chromatographic determination. Organic acids were separated using a reversed-phase column (Spherisorb S50DS2, 250 x 4 mm; PhaseSeparations, UK). The mobile phase, a 50 mmol potassium phosphate buffer, pH 2.4, was isocratically pumped at a flow rate of 1 ml/min, and the column temperature was set at 30°C. Relative retention values (retention factor, k) for lactic acid and acetic acid were 1.3 and 1.4, respectively.

Acetic acid and lactic acid were detected at a wavelength of 214 nm and quantitated using an external standard method. The relative standard deviation of repeated DL-lactic acid and acetic acid measurements derived from an in-house control sample (n = 5) were 11.0% and 24.8%, respectively. Peak identification was based on the retention time in the chromatogram and spiking of the sample extract with standard compound. Recovery values were calculated according to AOAC (1990). Recovery values of an added standard (n = 3) were 70.6% and 72.5% for lactic acid and acetic acid, respectively.

Microbiological determinations

Each experimental series was examined microbiologically after preparation (0 days) and after 4, 10 and 21 days of fermentation and 2, 3 and 5 months of storage. The following determinations were performed: total count of aerobically growing bacteria (APT agar / pH 7.0, BBL10918, 4 days at 30°C), LAB (APT agar / pH 5.6, BBL10918, 4 days at 30°C), staphylococci (Baird-Parker agar, Labm lab 85 and X085, 2 days at 37°C), pseudomonads (GSP, glutamate-starch-phenolred, agar, Kielwein 1969, 4 days at 25°C) and yeast and moulds (Rose-Bengal agar, Labm lab 36 and X009, 2–4 days at 30°C).

The type of predominating LAB was confirmed by examining microscopically three colonies of predominating colony type/types from APT agar / pH 5.6. The predominating LAB type/types are presented in the Table 3 behind the LAB counts.

Sensory evaluations

The total palatability of 1-, 2-, 4- and 5-month-old cabbage was evaluated by comparing them to a 3-month-old commercial product, using a paired test. The evaluation was performed by a panel of 5–7 persons familiar with sensory evaluation of foodstuffs.

Statistical methods

The results of pH, titrated acid and sugar determinations were subjected to statistical tests (multivariate analysis/StatGraphics win 32).

Results and discussion

The pH value and titrated acid

The mean pH value of cabbage juice measured immediately after preparation ranged from 5.84 to 6.20 (Table 1). The pH decreased during the first 10 days of fermentation to values below pH 4, which is near the final values. This pH level is comparable to those announced for sour vegetables in the literature (ICMSF 1980). *P. pen-*

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Table 1. The pH value of experimental sour cabbage groups (1–6) during fermentation (0, 10 and 21 days) and storage (2, 3 and 5 months). The number of experimental series = 3.

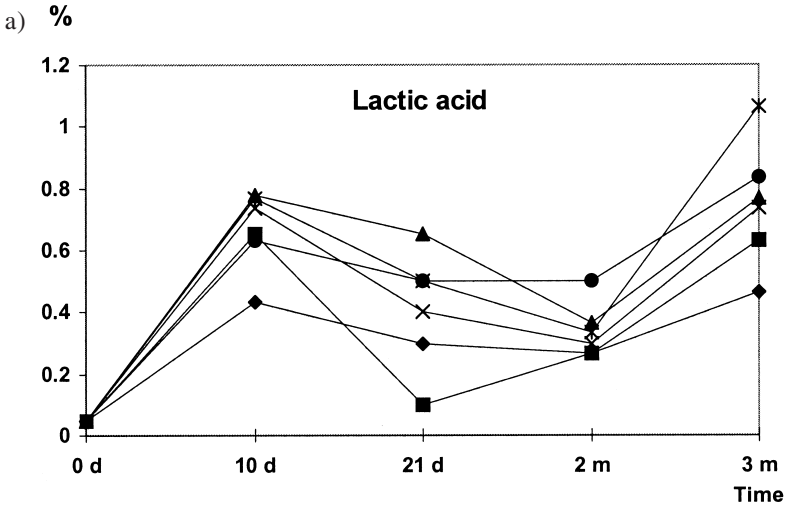
Cabbage group	0 days	10 days	21 days	2 months	3 months	5 months
1. Control						
x	6.18[ab]	3.87[a]	3.90[a]	3.97[a]	3.94[a]	3.62[a]
s	0.10	0.07	0.05	0.06	0.05	0.20
2. Lactob.						
x	5.84[a]	3.86[a]	3.89[a]	3.97[a]	3.88[a]	3.58[a]
s	0.10	0.07	0.05	0.06	0.05	0.20
3. Lactob. + Ped.						
x	6.03[ab]	3.62[b]	3.57[b]	3.61[b]	3.60[b]	3.32[b]
s	0.10	0.07	0.05	0.06	0.05	0.20
4. Lactob. + yeast						
x	5.98[ab]	3.55[b]	3.50[b]	3.63[b]	3.56[b]	3.29[b]
s	0.10	0.07	0.05	0.06	0.05	0.20
5. Ped.						
x	6.02[ab]	3.48[b]	3.54[b]	3.61[b]	3.57[b]	3.28[b]
s	0.10	0.07	0.05	0.06	0.05	0.20
6. Ped. + yeast						
x	6.20[b]	3.51[b]	3.55[b]	3.64[b]	3.57[b]	3.18[b]
s	0.10	0.09	0.06	0.07	0.06	0.24

x = mean
s = standard deviation of mean
Means within the vertical line not followed by the same small letter are significally different ($P<0.05$). If there are no letters after the means listed there are no differences among them.
1. Control, no inoculation
2. *L. alimentarius*
3. *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4. *L. alimentarius* + *C. sake* yeast strain
5. *P. pentosaceus* strain POHK
6. *P. pentosaceus* strain POHK + *C. sake* yeast strain

tosaceus strain POHK decreased the pH to 3.5 which is significantly ($P<0.05$) lower than fermentations using *L. alimentarius*, which did not decrease pH more than did wild LAB in control cabbage groups. This is in agreement with the fact that pediococci are active at lower pH values than lactobacilli (ICMSF 1980).

In experimental series III the pH values decreased to levels under pH 3.5 in the cabbage groups containing pediococci as inoculum, which confirms the strong acid-producing capacity of pediococci. At low pH the yeasts grew in series III. These results are sporadical, but indicate that pediococci as fermentation starter LAB may lead to growth of yeasts when they are no more competitive at low pH.

Frazier (1958) observed that the acid percentages of sour cabbages rose from 0.7% (wt/vol) to 2.0% during fermentation achieved with natural LAB flora. The range of titrated acid obtained in experimental sour cabbages immediately after preparation (0 days) was 0.4–0.9%. During the 10-day fermentation the acid content increased in inoculated cabbage groups to over 1.2%, with the highest amounts in those groups inoculated with *P. pentosaceus* strain POHK with or without yeast inoculum (range 1.4–1.7%). The content of titrated acid increased slowly there-



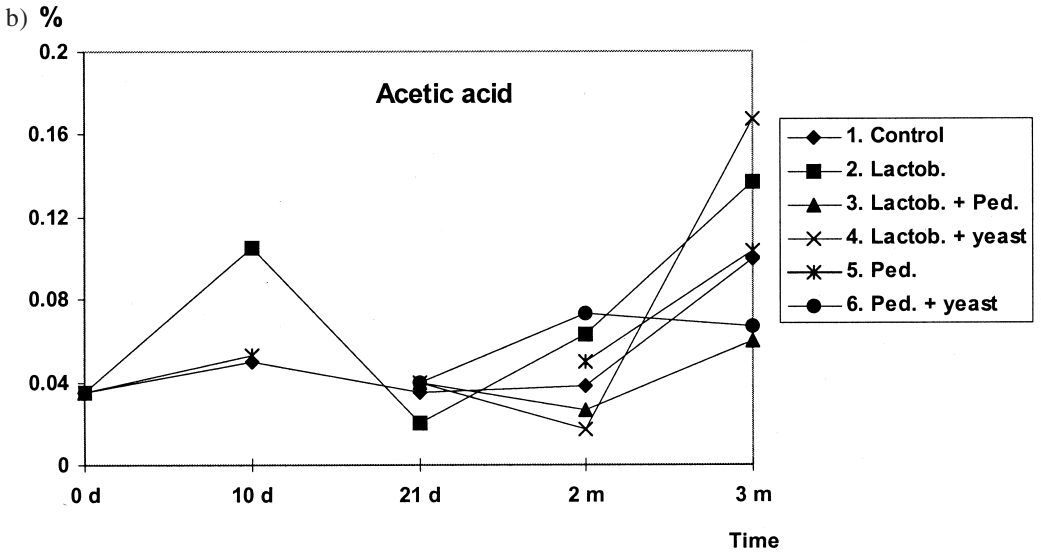


Fig. 1. Content (%; wt/vol) of lactic acid (a) and acetic acid (b) in experimental sour cabbage groups (1–6) during fermentation (0, 10 and 21 days) and storage (2 and 3 months). The number of experimental series = 3.

1 Control, no inoculation
2 *L. alimentarius*
3 *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4 *L. alimentarius* + *C. sake* yeast strain
5 *P. pentosaceus* strain POHK
6 *P. pentosaceus* strain POHK + *C. sake* yeast strain

after with the highest amounts appearing in POHK groups, (range 2.2–2.5%) 5 months after preparation. In noninoculated cabbage groups the content of titrated acid rose, but remained near 1% during the fermentation and storage periods. The titrated acid content of noninoculated cabbages and cabbages inoculated with *L. alimentarius* were significantly lower than cabbages with predominating pediococci but only 5 months after preparation.

Organic acids

The content of liquid-phase lactic acid and acetic acid immediately after preparation of sour cabbage batches ranged from 0.03 to 0.05 % (wt/vol) (Fig. 1). Most acid formation occurred during the first 10-day period; the amount of lactic acid was 0.4–0.8% after the 10-day fermentation period and was usually maintained at that level (Fig. 1a). Using *P. pentosaceus* POHK

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starter inoculation the formation of lactic acid was continued for a longer period, and the acid level attained values of 0.7–1.1% during 3 months of fermentation and storage. The lower acidity level was obtained with *L. alimentarius* inoculation, although lactobacilli were replaced by pediococci during fermentation. A rise in acetic acid content was observed during the first 10-day fermentation period, the contents increasing only slightly thereafter (Fig. 1b). The differences in overall acetic acid contents formed by various inoculation combinations were smaller than those of lactic acid.

Redox potential and a_w value

The redox potential of experimental sour cabbages ranged from +50 to +250 mV after preparation. The values decreased during fermentation and storage, but remained distinctly positive 5 months after preparation.

The a_w value of experimental sour cabbages ranged from 0.975 to 0.985 after 10 and 21 days of fermentation.

Sugars

The liquid phase of sour cabbage before fermenting (0 days) contained 0.6% (wt/vol) sucrose, 0.6% glucose and 0.4% fructose (Table 2). Rastas et al. (1989) observed the following contents of these sugars in cabbage: glucose no information, 0.1% saccharose and 0.4% fructose. Frazier (1958) reported higher sugar contents for cabbage, ranging from 2.9% to 6.4%. During the 10-day fermentation period the content of sucrose rose to 1–2%, decreasing thereafter to counts that were mostly under 0.9% 2 months after preparation. In our study differences ($P<0.05$) among cabbage groups were not observed.

Glucose contents rose during fermentation due to the splitting of sucrose; the highest values (about 1%) were obtained after 21 days of fermentation (Table 2). During storage the glucose contents did not decrease noticeably. The differences ($P<0.05$) among cabbage groups were not observed.

The fructose contents rose to their highest levels (1–2%) during 10 days of fermentation, but decreased during the remaining period of fermentation and storage (Table 2). The differences ($P<0.05$) among cabbage groups were not observed.

The contents of all 3 sugars increased during fermentation, due to gradual removal of the sugars from the cabbage to the liquid phase. According to Buckenhüskes (1993) sugars should be removed during fermentation to prevent yeast growth in sour cabbage.

Lactic acid bacteria

The LAB count corresponded to the total count of bacteria in all samples. The LAB counts ranged from 6.2 to 7.4 log cfu/g after inoculation, increasing by 0.0–1.9 log cfu/g during the first 10 days of fermentation (Table 3). During this time the pH decreased to levels under pH 4. During the next 11 days the LAB counts decreased by 0.2–3.8 log cfu/g in 17 batches out of 18; this decrease continued mostly during the following 4 months. The counts varied from <2 to 6.5 log cfu/g in experimental products 5 months after preparation, suggesting that LAB may disappear completely from the product during storage.

Lactobacilli and pediococci formed the predominant part of the microbial flora in sour cabbages prepared without bacterial inocula or with *L. alimentarius* inocula (Table 3). The predominant type varied among experimental series. When *L. alimentarius* was used with *P. pentosaceus* strain POHK or *C. sake* yeast as inocula pediococci already displaced lactobacilli during the first 10 days of fermentation, which agrees with earlier information on the development of bacterial flora in fermented vegetables

Table 2. Content (%) of sucrose, glucose and fructose in experimental sour cabbage groups (1–6) during fermentation lasting 10 and 21 days (d) and storage lasting 2 and 5 months (m). Contents (%) at the beginning of fermentation (0 days) were as follows: sucrose 0.6, glucose 0.6 and fructose 0.4. The number of experimental series = 3.

	Sucrose				Glucose				Fructose			
Cabbage group	10 d	21 d	2 m	5 m	10 d	21 d	2 m	5 m	10 d	21 d	2 m	5 m
1. Control												
x	1.6	1.0	0.2	0.8	1.1	1.1	0.4	0.8	1.9	1.1	0.4	0.8
s	0.1	0.7	0.0	0.1	0.4	0.2	0.4	0.6	0.1	0.8	0.4	0.9
2. Lactob.												
x	0.9	0.9	0.3	0.1	0.9	1.1	0.7	0.9	1.9	1.3	0.4	0.5
s	0.4	0.2	0.0	0.1	1.0	0.3	0.1	0.7	0.1	0.4	0.2	0.4
3. Lactob. + Ped.												
x	1.3	0.9	0.9*	0.1	1.0	0.9	0.6*	0.7	1.6	1.6	0.6*	1.0
s	0.4	0.2		0.1	0.3	0.4		0.8	0.6	0.5		0.7
4. Lactob. + yeast												
x	1.4	0.9	0.1	0.2	1.0	0.8	0.7	1.1	1.6	1.4	0.4	0.9
s	0.2	0.3	0.0	0.1	0.1	0.6	0.9	0.1	0.6	0.2	0.4	0.5
5. Ped.												
x	1.3	0.6	0.5	0.4	0.9	1.6	0.9	0.9	1.6	0.6	0.7	0.8
s	0.6	0.2	0.1	0.3	0.5	0.8	0.6	0.6	0.6	0.7	0.1	0.7
6. Ped. + yeast												
x		1.8	1.9*	0.6		1.4	1.9*	1.0		1.4	0.8*	1.1
s		0.8		0.5		0.2		0.7		0.7		0.1

* only one value

x = mean

s = standard deviation of mean

Means within the vertical line not followed by the same small letter are significally different ($P < 0.05$). If there are no letters after the means listed there are no differences among them.

1. Control, no inoculation
2. *L. alimentarius*
3. *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4. *L. alimentarius* + *C. sake* yeast strain
5. *P. pentosaceus* strain POHK
6. *P. pentosaceus* strain POHK + *C. sake* yeast strain

(ICMSF 1980). When *P. pentosaceus* POHK alone was used as an inoculum, it remained the predominant form of LAB.

In experimental series III, yeasts appeared as the predominant microbes after 2 months of fermentation in all inoculated experimental groups, which can be explained by the low pH values (<3.5) encountered in this series. This would indicate that at low pH even pediococci do not survive and that yeasts begin to dominate the microbial flora.

Yeasts

In the sour cabbage groups inoculated with *C. sake* yeast strain, the yeast counts did not rise during fermentation and storage (Table 4). On the other hand, in cabbage groups prepared without yeast inocula, yeasts appeared almost regularly. In experimental series III, yeasts formed the predominant microbial group in inoculated cabbages 2 months after preparation. On the basis of these observations, it can be concluded that

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Table 3. Lactic acid bacterial counts (log cfu/g) of experimental sour cabbage groups (1–6) on APT agar (pH 5.6) during fermentation (0, 10 and 21 days) and storage (2, 3 and 5 months). The counts of different experimental series (I–III) are presented separately. The pH values after 10 days of fermentation are also presented.

Cabbage group	0 days	10 days	pH 10 days	21 days	2 months	3 months	5 months
1. Control							
I		7.9 s	3.86	6.3 s	6.2 l	5.2 l, p	4.3 l
II		8.6 l, (p)	3.80	8.2 p	6.5 p, (l)	6.7 l, (p)	6.8 p, (l)
III	5.2 l (p)	5.6 l	3.96	5.1 p, (l)	4.6 p	5.1 p	6.5 p
2. Lactob.							
I	7.3 l	8.4 p, (l)	3.74	5.8 l	5.5 l	4.4 l, (p)	4.6 p
II	6.7 l	8.6 p, (l)	3.90	6.4 p	8.2 p, (y)	7.8 p, (y)	7.4 p
III	6.4 l	< 5	3.92	4.1 l	6.3 l, (y)	3.0 y	7.5 y
3. Lactob. + Ped.							
I	6.5 p, 7.0 l	8.0 p	3.65	7.8 p	6.9 p	4.0 p	< 2
II	6.8 p, 6.9 l	7.9 p	3.74	7.3 p	5.4 p	3.8 p	6.5 p
III	7.5 l	7.6 p	3.46	4.6 y	7.3 y	6.6 y	6.9 y
4. Lactob. + yeast							
I	7.0 l	7.3 p	3.46	6.1 p	5.8 p	3.9 p	4.0 p, (y)
II	6.4 l	6.9 p	3.76	7.4 p	5.9 p	4.3	3.6 p, (y)
III	7.4 l	7.3 p	3.44	5.5 p, (y)	5.5 y	6.0 y	6.6 y
5. Ped.							
I	6.8 p	8.5 p, (l)	3.39	6.4 p, (l)	4.0 p	5.9 p	2.3 l
II	7.2 p	8.4 p, (l)	3.57	6.2 p	6.5 p, (y)	6.5 p, (y)	4.5 y, (p)
III	6.2 p	7.6 p	3.47	4.0 p	2.3 p, (y)	2.5 l	4.6 y
6. Ped. + yeast							
I							
II	7.2 p	7.5 p	3.53	6.8 p	4.5 p, (y)	4.5 p, (y)	< 2
III	6.2 p	8.0 p	3.50	4.2	6.3	5.0 y	6.7 y

s = streptococci
p = pediococci
l = lactobacilli
y = yeast
( ) = secondary microbial group
1. Control, no inoculation
2. *L. alimentarius*
3. *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4. *L. alimentarius* + *C. sake* yeast strain
5. *P. pentosaceus* strain POHK
6. *P. pentosaceus* strain POHK + *C. sake* yeast strain

yeasts may appear in sour vegetables at least when ripened and stored 2 months or more. They may even form the predominant part of the microbial flora. Buckenhüskes (1993) suggested that all sugars should be removed during fermentation to prevent yeast growth; however, those amounts of sugars present in the sour cabbages after fermentation in this investigation did not appear to enhance the growth of yeasts in the present study.

Table 4. Yeast counts (log cfu/ g) of experimental sour cabbage groups (1–6) on Rose-Bengal agar during fermentation (0, 10 and 21 days) and storage (2, 3 and 5 months). The counts of different experimental series (I–III) are presented separately. The pH values after 10 days of fermentation are also presented.

Cabbage group	0 days	10 days	pH 10 days	21 days	2 months	3 months	5 months
1. Control							
I		3.2	3.86	< 2	< 2	< 2	2.6
II	< 2	< 2	3.80	2.0	< 2	< 2	< 2
III	3.0	3.6	3.96	< 2	< 2	< 2	3.7
2. Lactob.							
I		5.3	3.74	< 2	< 2	< 2	< 2
II	2.3	4.3	3.90	5.2	5.6	5.0	5.7
III	2.5	3.7	3.92	2.8	< 2	< 2	7.0
3. Lactob. + Ped.							
I	3.7	3.2	3.65	< 2	< 2	< 2	< 2
II	< 2	< 2	3.74	< 2	2.3	< 2	< 2
III	4.3	3.6	3.46	< 2	6.0	5.9	6.8
4. Lactob. + yeast							
I	3.7	2.8		3.3	< 2	< 2	3.4
II	3.7	2.0	3.76	3.0	2.3	< 2	2.9
III	4.0	3.9	3.44	3.6	5.5	4.7	4.5
5. Ped.							
I	3.5	5.6	3.39	< 2	< 2	< 2	< 2
II		5.2	3.57	4.8	3.9	5.0	6.4
III	3.9	3.8	3.47	< 2	< 2	2.0	4.5
6. Ped. + yeast							
I							
II	3.7	< 2	3.53	< 2	2.0	< 2	< 2
III	4.2	4.0	3.50	4.2	6.0	4.6	6.3

1. Control, no inoculation
2. *L. alimentarius*
3. *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4. *L. alimentarius* + *C. sake* yeast strain
5. *P. pentosaceus* strain POHK
6. *P. pentosaceus* strain POHK + *C. sake* yeast strain

Staphylococci and pseudomonads

The staphylococcal and pseudomonad counts decreased to <2 log cfu/g during the first 10 days of fermentation.

Sensory evaluation

Commercial sour cabbage (3 months old) proved significantly better than 1-, 2- and 4-month-old experimental cabbages when the paired test figures of different cabbage groups were summed and the sums were compared using paired tests (Table 5). Five-month-old cabbages were not significantly worse than the commercial product when compared in the manner described.

The results can be summarized as follows

The counts of inoculated LAB were on the level of 7.0 log cfu/g increasing by 0.0–1.9 log cfu/g during the first 10 days of fermentation. The pH

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Table 5. Sensory evaluation of total palatability of experimental sour cabbages by comparing cabbages of different ages to reference cabbage (3-month-old commercial product). The figures are the numbers of better evaluations of paired comparisons.

Cabbage group	1 m – 3 m		2 m – 3 m		4 m – 3 m		5 m – 3 m	
1. Control	0	7	2	5	1	4	3	2
2. Lactob.	1	6	2	5	1	4	0	5
3. Lactob. + Ped.	2	5	2	5	1	4	1	4
4. Lactob. + yeast	2	5	2	5	1	4	2	3
5. Ped.	1	6	2	5	1	4	2	3
6. Ped. + yeast	1	6	1	6	1	4	3	2
Total	7	35 *	11	31 *	6	24 *	11	19

* significantly better in paired comparison
m = months
1. Control, no inoculation
2. *L. alimentarius*
3. *L. alimentarius* + *P. pentosaceus* strain POHK (from sour carrot strips)
4. *L. alimentarius* + *C. sake* yeast strain
5. *P. pentosaceus* strain POHK
6. *P. pentosaceus* strain POHK + *C. sake* yeast strain

decreased during that time to levels under 4, which is near the final values. Pediococci formed predominant part of microbial flora almost in all experimental batches. Pediococci fermented cabbage effectively, decreasing the pH to lower levels than lactobacilli or natural LAB flora of control group. The counts of LAB, also pediococci, decreased after 10 days of fermentation. The low pH and decreasing of LAB counts enhanced the growth of yeasts in some cases.

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SELOSTUS

Maitohappobakteerien hyödyntäminen hapankaalin fermentoinnissa

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Hapanvihannesten fermentointi on tapahtunut perinteisesti vihannesten omien maitohappobakteerien (MHB) avulla. Monissa muissa hapatetuissa elintarvikkeissa fermentointi tapahtuu valmisteeseen lisättyjen MHB:n avulla. Tämä artikkeli käsittelee tutkimusta, jossa selvitettiin hapankaalin fermentointia tiettyjen MHB-siirrostuksien (*Lactobacillus alimentarius* ja *Pediococcus pentosaceus*) avulla. Koska hiivojen esiintyminen hapanvihanneksissa on ollut ongelma, tutkittiin myös MHB-siirrostuksien vaikutusta hiivojen kasvun estäjänä.

Kaalimehun pH laski kymmenen ensimmäisen fermentointipäivän aikana alle neljän, eli lähes lopulliselle pH-tasolle. Inokuloidut pediokokit laskivat pH-arvoa eniten. Myös korkeimmat titratun hapon pitoisuudet, 2,2–2,5 % (paino/tilavuus) viisi kuukautta valmistuksen jälkeen, esiintyivät pediokokeilla inokuloiduissa kaaleissa. Sokerien määrä lisääntyi kaalimehussa fermentoinnin aikana johtuen niiden asteittaisesta siirtymisestä kaalisolukosta nesteosaan. Inokuloitujen MHB:n lukumäärä kaalimehussa kasvoi 0,0–1,9 log pmy (pesäkkeen muodostava yksikkö)/g kymmenen ensimmäisen fermentointivuorokauden aikana laskien sen jälkeen. Pediokokit olivat valtaorganismeja lähes kaikissa kaalierissä. Hiivoilla inokuloitujen kaalien hiivapitoisuus nousi vain kun pH-arvo oli alle 3,5. Hiivoja esiintyi usein myös kaalierissä, joita ei oltu inokuloitu hiivoilla.

Tutkimus osoitti, että hapankaalia voidaan valmistaa fermentoimalla kaali valituilla maitohappobakteereilla. Pediokokit fermentoivat kaalia tehokkaasti laskien pH-arvon alemmaksi kuin laktobasillit tai kontrolliryhmän MHB-floora. Seurauksena voi kuitenkin olla liian voimakas haponmuodostus ja pH-arvon lasku, jotka johtavat maitohappobakteerimäärän vähenemiseen. Tämä edistää hiivojen kasvua. Hiivojen esiintymisongelmaa ei siten voitu ratkaista pelkällä maitohappobakteerilisäyksellä.