

Effect of Starter Cultures *L. mesenteroides* and *L. lactis* ssp. *lactis* on Sauerkraut Fermentation and Quality

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Abstract

KRISTEK S., BEŠLO D., PAVLOVIĆ H., KRISTEK A. (2004): **Effect of starter cultures *L. mesenteroides* and *L. lactis* ssp. *lactis* on sauerkraut fermentation and quality.** Czech J. Food Sci., 22: 125–132.

Sauerkraut fermentation course was observed in 3 cycles and 4 replicates under controlled conditions (2.5% NaCl, 21°C) using starter cultures (control; *Leuconostoc mesenteroides* – 700 mil. cfu/ml; *Lactococcus lactis* ssp. *lactis* – 500 mil. cfu/ml; preceding fermentation juice). Each of the above mentioned cycles lasted for 28 days. Microbiological and chemical characteristics of the sauerkraut during the 28-day fermentation period were investigated. Lactic acid bacteria content, the concentration of lactic acid, and pH of the medium were monitored daily. The completion of each cycle fermentation was followed by the determination of the final product organoleptic properties which were observed for 6 months with the aim to define the expiration date. The best results relative to sauerkraut quality were obtained by using starter culture *L. lactis* ssp. *lactis*, followed by fermentation conducted by natural, spontaneous sauerkraut flora (control variant). Organoleptic properties and expiration date of the final product obtained by the use of lactic acid bacterium *L. mesenteroides* as a starter culture were better compared to the use of sauerkraut juice obtained from the preceding fermentation cycles and possessing the best organoleptic properties.

Keywords: fermentation; lactic acid bacteria; *L. lactis* ssp. *lactis*; *L. mesenteroides*; organoleptic properties; sauerkraut

Apart from potatoes, sauerkraut is the most dominant foodstuff in Croatia. The nutritional value of this tasteful and well protected cabbage form is very high. Namely, C vitamin content is equal to that of the lemon. Furthermore, NONNECKE (1989) reported that it is also a source of minerals and vitamins including calcium, phosphorus, iron, sodium, potassium, vitamin A, thiamine, riboflavin, niacin (and ascorbic acid). VON WIMPFEN (1989) reported that fermented cabbages have high contents of vitamins B and C compared to not fermented cabbages.

Sauerkraut is a product of lactic acid fermentation spontaneously developed by lactic acid bacteria under anaerobic conditions with the use of a certain

amount of salt (NaCl) at optimal temperatures. An adequate sequence of the fermentation process bacteria is necessary to inhibit undesired rot-causing microorganisms development. Optimal temperatures (20–22°C) and NaCl level (1.0–2.5%) were determined by PARMELE *et al.* (1927) and MARTEN *et al.* (1929). In Croatia, sauerkraut fermentation, also in commercial production relies only on the natural population of the fresh cabbage bacteria. This is the reason why the quality of sauerkraut varies in the production cycles rarely resulting in the achievement of equal quality of the products.

Apart from the temperature and salt concentration, the final product quality depends on the cabbage variety, i.e. its chemical composition.

KYUNG and FLEMING (1994) stated that some cabbage components show antibacterial effects on some lactic acid bacteria strains, and that their concentration varies in some varieties. The cooler northern regions of Europe prefer the light-headed white cabbage whereas in the warmer southern regions the looser-heading type known as *romano* is preferred (NONNECKE 1989).

The introduction of starter cultures would result in the control of the production process and the achievement of the desired product quality in each cycle regardless of the natural bacteria population in the fresh cabbage (HARRIS *et al.* 1992; BREIDT *et al.* 1995; KALAČ *et al.* 2000).

HARRIS *et al.* (1992) and BREIDT *et al.* (1993, 1995) used bacterium *L. mesenteroides* as a starter culture in sauerkraut fermentation and obtained sauerkraut characterised by better organoleptic properties compared to the control variant. HARRIS *et al.* (1992) used homofermentative lactic acid bacteria – *L. brevis* and *Lb. plantarum* as the starter cultures and obtained a product of darker colour and wine vinegar-like bitter taste. LÜCKE *et al.* (1990) used *Lb. bavaricus* for sauerkraut fermentation and achieved a product marked for its perfect organoleptic properties.

MATERIAL AND METHODS

A Holland variety Quisto F₁ (selection – house is Sluis en Groot) was used in the experiments. Quisto F₁ is a variety characterised by dark green round heads weighing about 3 kg. Heads are hard and crack-resistant. Apart from the domestic variety Čepinski cabbage, Quisto F₁ is the most preferred by the local consumers. The test was conducted in three fermentation cycles and 4 replicates. The first cycle comprised by three variants: 1st – control, 2nd – application of the starter culture *Leuconostoc mesenteroides* (700 mil. cfu/ml), 3th – application of the starter culture *L. lactis* ssp. *lactis* (500 mil. cfu/ml). The second and third cycles were characterised by the introduction of the 4th variant – juice obtained from the preceding fermentation showing the best organoleptic properties of sauerkraut.

The washing and rinsing of the cabbage heads were followed by placing the heads into barrels of 100 litres. Salt solution (2.5%) was prepared which was then boiled and cooled. Depending on the variant, the respective, starter culture (pure cultures, freeze – dried, Direct Vat Set, Christian Hansen, Denmark) was added, and the resulting

solution was poured into the barrels until the heads were completely covered. The barrels were then closed with lids.

Fermentation developed in the conditioned chamber at 21°C and lasted for 28 days. After the fermentation was terminated, sauerkraut was stored at the temperature of 10°C. Lactic acid content was determined daily by HPLC method (SNYDER 1997) and the acidity by pH meter. The isolation of bacteria was performed by the routine microbiological procedure, and inoculation on a solid medium. The number of homo-fermentative bacteria *L. lactis* ssp. *lactis* was determined on HHD broth (Biolife). Typical formula HHD broth: Fructose 2.500 g/l, Sucrose 0.200 g/l, Monopotassium Phosphate 2.500 g/l, Tryptic Digest of Casein 10 000 g/l, Soy Peptone Bios 1.500 g/l, Hydrolysed Casein Bios 3.000 g/l, Yeast Extract 1.000 g/l, Bromocresol Green 0.066 g/l, and Tween 80 (1 g/l). The number of hetero-fermentative bacteria *L. mesenteroides* was determined on the selective medium LUSM. This medium contained 1.0% glucose, 1% Bacto Peptone (Difco), 0.5% yeast extract (BBL), 0.5% meat extract (Difco), 0.25% Gelatin (Difco), 0.5% calcium lactate, 0.05% sorbic acid, 75 ppm of sodium azide (Sigma), 0.25% sodium acetate, 0.1% (vol/vol) Tween 80, 15% tomato juice, 30 µg of Vancomycin (Sigma) per ml, 0.20 µg of Tetracycline (Serva) per ml, and 1.5% agar (Difco). The number of bacteria *Lactobacillus cucumeris* was determined on selective medium MRS AGAR (DE MAN *et al.* 1960) which contained: peptone 10.0 g/l, Lab-Lemco powder 8.0 g/l, yeast extract 4.0 g/l, glucose 20.0 g/l, sorbitan mono-oleate 1 ml, dipotassium hydrogen phosphate 2.0 g/l, sodium acetate × 3H₂O 5.0 g/l, triammonium citrate 2.0 g/l, magnesium sulphate × 7H₂O 0.2 g/l, manganese sulphate × 4H₂O 0.05 g/l, agar 10.0 g/l.

The determination of the strains was performed on the basis of their morphological, cultural, physiological and biochemical characteristics by the procedures described in KRIEG and HOLT (1984).

The completion of fermentation was followed by the determination of the organoleptic properties of the fermented products which were monitored for 6 months with the aim to define the expiration time.

The results were processed by modern statistical methods (ANOVA) using the computer program by StatSoft Inc. (2001) Statistica (data analysis software system), version 6.

RESULTS AND DISCUSSION

Control – spontaneous fermentation

The initial fermentation phase was characterised by the markedly dominant hetero-fermentative bacterium *L. mesenteroides*. This is in harmony with the statements by MUNDT (1970), FLEMING *et al.* (1988) and AXELSSON (1998) who reported that fermentation was initiated by these bacteria due to their dominance in sauerkraut. Their presence at the beginning of the fermentation is the evidence that the fermentation develops properly. SCHLEGEL (1992) reported that hetero-fermentative phase was characterised by the production of lactic acid, acetic

acid, ethyl alcohol, and CO₂. Compounds derived from this phase are responsible for the final product taste and flavour. Taking into account the mean content of *L. mesenteroides* being 2.2×10^2 cfu/ml (0 fermentation day), the number of these bacteria was increasing up to 5th fermentation day (5.6×10^7 cfu/ml) and decreasing afterwards. Tenth day showed no presence of these bacteria in the medium (Figure 1). McDONALD *et al.* (1990) reported that the rapid decay of these medium bacteria is the results of their sensitivity to the acid conditions. The pH value on the last fermentation day was 3.66–3.78 (Table 1) whereas the mean lactic acid content was 1.78% (Figure 2). Total number of the lactic acid

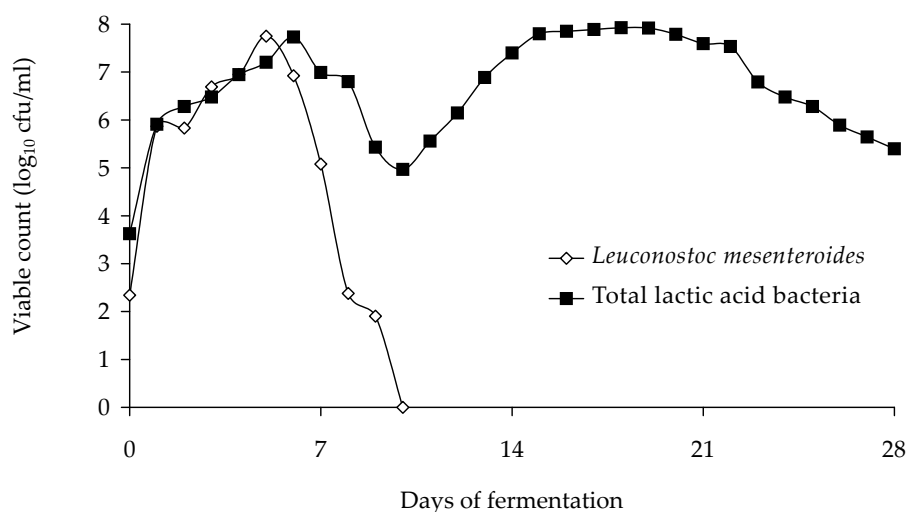


Figure 1. Changes in *Leuconostoc mesenteroides* and total lactic acid bacteria count during fermentation of control sauerkraut

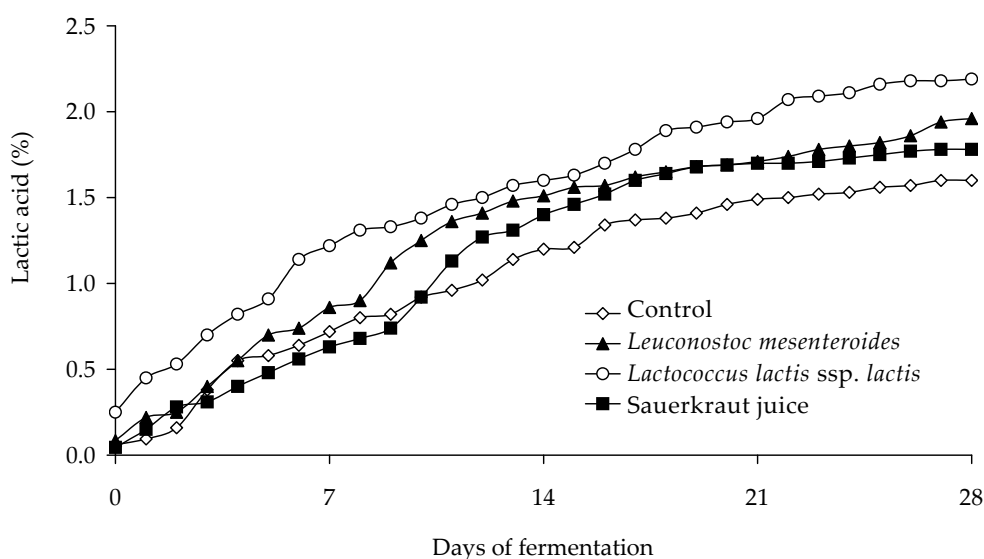


Figure 2. Percent of lactic acid produced during fermentation of sauerkraut inoculated with different starter cultures

Table 1. pH values of the investigated variants and LSD

Day	Control A			<i>Leuconostoc mesenteroides</i> B			<i>Lactococcus lactis</i> ssp. <i>lactis</i> C			Cabbage juice D	
				Cycle							
	I	II	III	I	II	III	I	II	III	II	III
0	6.20	6.03	6.28	6.12	6.00	6.02	6.01	5.98	6.06	5.35	5.50
1	5.98	5.78	5.92	5.90	5.78	5.82	5.66	5.50	5.58	5.11	5.29
2	5.34	5.41	5.54	5.56	5.44	5.50	5.24	5.18	5.10	4.77	5.01
3	4.80	5.08	5.26	5.12	5.28	5.32	5.08	5.08	4.76	4.48	4.83
4	4.46	4.62	4.80	4.92	4.86	5.04	4.80	4.90	4.60	4.21	4.61
5	4.35	4.21	4.48	4.88	4.60	4.75	4.44	4.75	4.32	4.06	4.29
6	4.22	4.16	4.30	4.52	4.34	4.52	4.16	4.46	4.14	3.82	3.99
7	4.18	4.08	4.20	4.40	4.30	4.28	4.03	4.22	3.99	3.68	3.78
14	3.91	3.88	3.92	4.12	4.10	4.08	3.80	3.87	3.71	3.31	3.46
21	3.82	3.74	3.72	3.95	3.91	4.01	3.62	3.72	3.53	3.20	3.33
28	3.78	3.69	3.66	3.88	3.86	3.90	3.52	3.58	3.44	3.15	3.22
LSD	Variants										
	A**			B			C**			D*	
0.05	0.059			n.s.			0.066			0.074	
0.01	0.082			n.s.			0.109			0.090	
										ABCD**	

n.s. – non significant

bacteria was 2.5×10^5 cfu/ml on the completion of fermentation. The final products in all three cycles revealed good organoleptic properties (Table 2) although sauerkraut had slightly more intensive odour after 3–5 months (depending on the cycle). One replicate (1st and 2nd fermentation cycle) was characterised by a markedly mucous sauerkraut caused by the medium presence of *Lactobacillus cucumeris* in the medium (1.2×10^4 cfu/ml; 3.7×10^4 cfu/ml).

Starter culture – *Leuconostoc mesenteroides*

Introduction of the starter culture *L. mesenteroides* in the fermentation process resulted in the final product whose mean acidity (lactic acid) was 1.60% (Figure 2) whereas pH ranged between 3.86–3.90 (Table 1). The introduced number of 7×10^8 cfu per ml increased to 1.6×10^9 cfu/ml (4th fermentation day) and then decreased to 0.4×10^2 cfu/ml on the 10th fermentation day (Figure 3). Such developing course of this bacterium is in accordance with

the findings made by STAMER *et al.* (1971) who reported that *L. mesenteroides* developed rapidly (compared to other bacteria), having shorter life cycle (generation time) than other sauerkraut juice lactic acid bacteria. In spite of rapidly becoming dominant *L. mesenteroides* decays fast as the fermentation proceeds, due to its sensitivity to the acid conditions (McDONALD *et al.* 1990). Namely, *L. mesenteroides* being a hetero-fermentative bacterium, it produces CO₂, ethyl alcohol, and also small amounts of lactic and acetic acids. When acidity reached over 0.9% (lactic acid) this medium bacterium almost disappeared. The final product achieved was of mild odour, slightly sour and of somewhat softer structure (Table 2). According to AXELSSON (1998), *L. mesenteroides* added in the preceding phase provides the final product with good flavour and changes natural bacteria sequence in the fermentation course. The aforesaid resulted in an incompletely fermented product. Some replicates of this variant were known for mucous sauerkraut incidence (3–8 weeks after the

Table 2. Organoleptic properties of the investigated variants (6 months after fermentation completion)

Organoleptic properties	Variant										
	A			B			C			D	
				cycle							
	I	II	III	I	II	III	I	II	III	II	III
Odor											
a – pleasant odor of sauerkraut							a ₄	a ₃	a ₃		
b – sharp odor of acetic acid	b ₁	b ₂	b ₂							b ₄	b ₃
c – very mild odor				c ₂	c ₄	c ₄					
Color											
a – straw yellow	a ₁	a ₃		a ₁	a ₂	a ₂	a ₃	a ₅	a ₄		
b – pale yellow			b ₃							b ₃	b ₄
c – brown											
d – pink											
Taste											
a – pleasant sour	a ₂	a ₃	a ₂				a ₅	a ₄	a ₄		
b – bitter										b ₂	b ₄
c – very mild sour				c ₃	c ₄	c ₃					
Texture											
a – hard							a ₃		a ₂		
b – semihard	b ₃		b ₁		b ₃			b ₅			
c – soft		c ₃		c ₂		c ₃				c ₄	c ₅

A – control; B – *Leuconostoc mesenteroides*; C – *Lactococcus lactis* ssp. *lactis*; D – cabbage juice (last fermentation cycle)
 1 – denotes the lowest pronounced property; 5 – denotes the highest pronounced property

completion of fermentation). BREIDT *et al.* (1993, 1995) reported that mucous incidence is the result of the saccharose-produced dextrane, which is a product of these bacteria metabolism. Total number of lactic acid bacteria on the last fermentation day was 2.1×10^6 cfu/ml.

Starter culture – *Lactococcus lactis* ssp. *lactis*

The best sauerkraut quality was achieved by introducing starter culture *L. lactis* ssp. *lactis* into the fermentation process. On the 7th fermentation day (initial fermentation was 5×10^8 cfu/ml), the number of these bacteria dropped to 0.3×10^2 cfu per ml and they were not found later in the medium (Figure 4). This is in accordance with the results of the investigation conducted by ANONYMUS (1993) and BATTCOCK and AZAM-ALI (1998) who reported that *L. lactis* ssp. *lactis* survived for a short period

only in the medium. However, its presence lasts long enough to initiate sauerkraut fermentation process without disturbing natural microorganisms sequence. The number of hetero-fermentative bacteria *L. mesenteroides* ranged from 1.2×10^2 (0 day) to 4.0×10^7 (5th fermentation day) and then decreased. Their number as well as the oscillation in the course of the fermentative cycle were similar as in the fermentation developed by a spontaneous sauerkraut microflora. This is in harmony with the data by HARRIS *et al.* (1992) who reported that the presence of the lactic acid bacteria *L. lactis* ssp. *lactis* did not affect the number of bacteria *L. mesenteroides* in the fermentation process. The reason for that is the resistance of the bacterium *L. mesenteroides* to nisine, bacteriocine produced by most *L. lactis* species. Total number of lactic acid bacteria was 6.4×10^6 cfu/ml on 28th fermentation day. The pH value of the final product (Table 1)

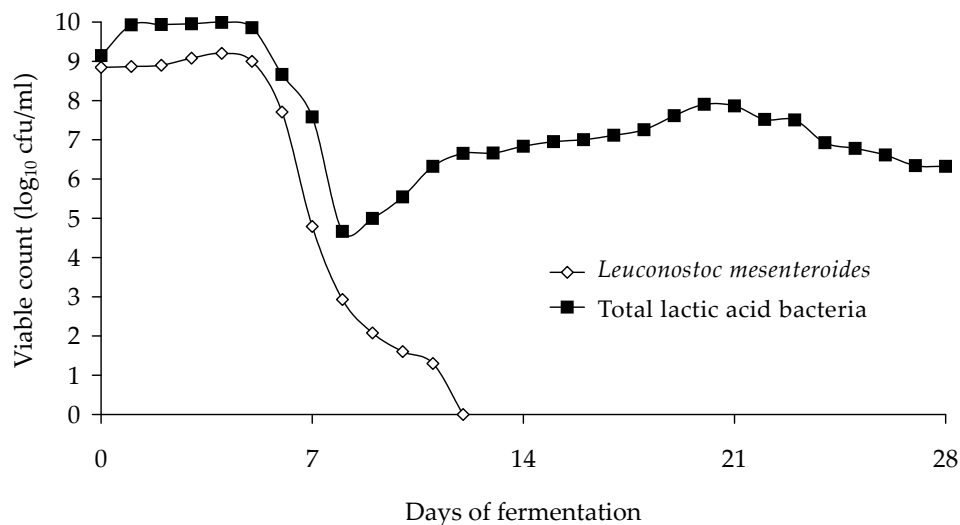


Figure 3. Changes in *Leuconostoc mesenteroides* and total lactic acid bacteria count during fermentation of sauerkraut inoculated with *Leuconostoc mesenteroides*

ranged between 3.44–3.58. Thus, sauerkraut was characterised by pleasant acid taste – 1.96% lactic acid (Figure 2). The products obtained in all production cycles possessed excellent organoleptic properties (Table 2) which did not change within 6 months of monitoring.

Sauerkraut juice

Both production cycles were characterised by the sauerkraut juice obtained from the preceding

fermentation where *L. lactis* ssp. *lactis* was used as the starter culture. The juice acidity was adjusted to 0.25% and NaCl was added to the concentration of 2.5%. Namely, ANONYMUS (1993) and AXELSSON (1998) stated that “old” juice efficiency depended on its microorganisms type and acidity. If the acidity is above 0.3%, cocca development is suppressed and the fermentation is developed by bacilli. In such case, the final product achieved is of low quality.

The number of bacteria *L. mesenteroides* was smaller in the hetero-fermentative phase of the ferment-

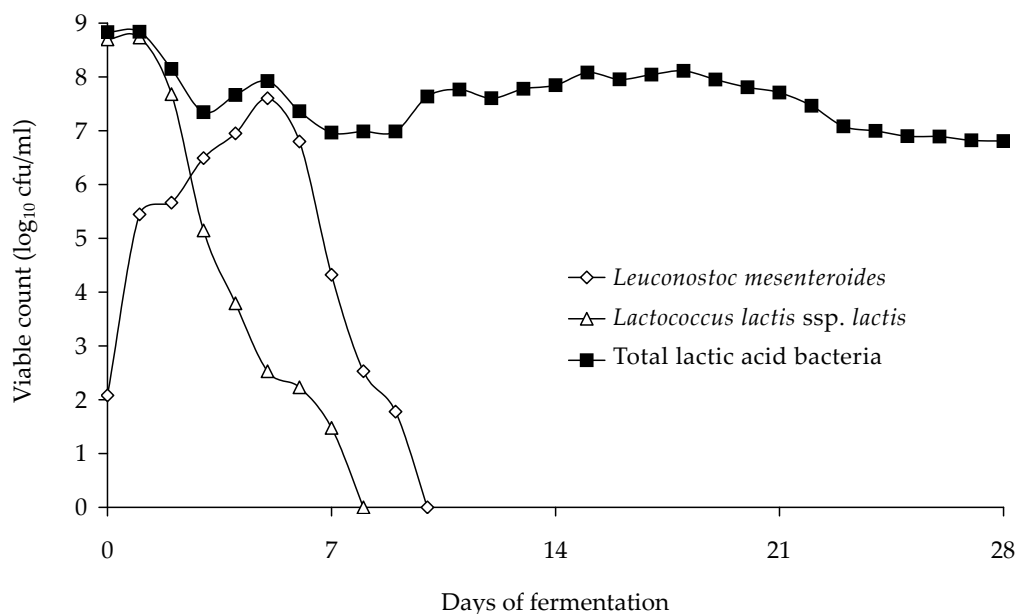


Figure 4. Changes in *Leuconostoc mesenteroides*, *Lactococcus lactis* ssp. *lactis* and total lactic acid bacteria count during fermentation of sauerkraut inoculated with *Lactococcus lactis* ssp. *lactis*.

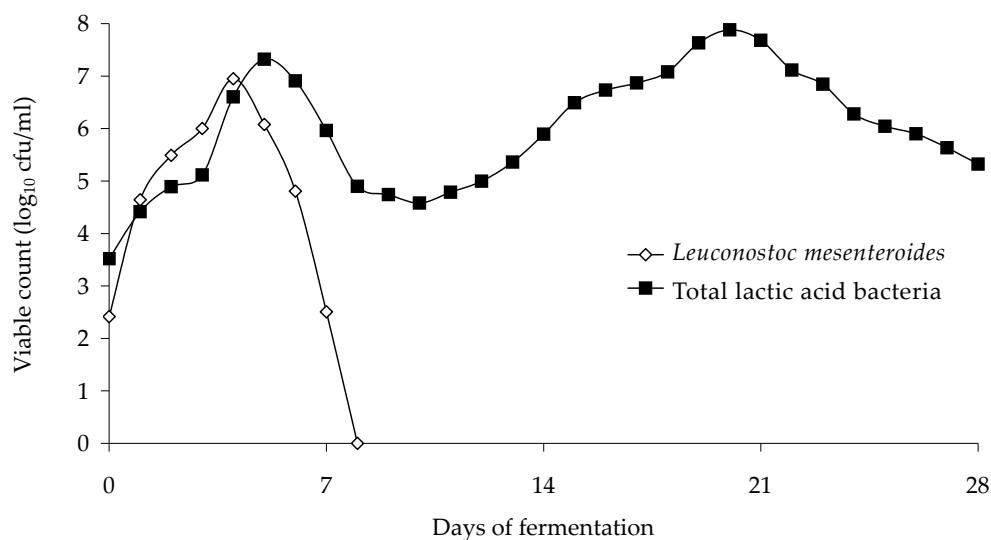


Figure 5. Changes in *Leuconostoc mesenteroides* and total lactic acid bacteria count during fermentation of sauerkraut inoculated with sauerkraut juice

tation. It ranged from 2.6×10^2 cfu/ml (0 day) to maximum of 8.9×10^6 cfu/ml (4th day). The medium was characterised by the absence of these bacteria on 8th fermentation day (Figure 5). It is obvious that this phase lasted for a shorter period compared to other variants resulting likely in considerably higher mean final acidity – 2.19% of lactic acid (Figure 2). The pH value was 3.15 in the first and 3.22 in the second cycle (Table 1). The sauerkraut achieved was marked by an intensive odour (acetic acid like), bitter taste, and softer structure (Table 2) compared to the products obtained with other variants. With the time, these properties became more pronounced.

CONCLUSION

The best results relative to the sauerkraut quality and expiration date were achieved with the variant using bacterium *L. lactis* ssp. *lactis* as the starter culture. This bacterium survived very shortly in the medium but long enough to initiate the fermentation process. The final product was of excellent organoleptic properties which remained unchanged within 6 months monitored.

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Received for publication April 13, 2004

Accepted after corrections July 19, 2004

Souhrn

KRISTEK S., BEŠLO D., PAVLOVIĆ H., KRISTEK A. (2004): **Vliv startovacích kultur *L. mesenteroides* a *L. lactis* ssp. *lactis* na fermentaci a kvalitu kyselého zelí.** *Czech J. Food Sci.*, **22**: 125–132.

Průběh fermentace kyselého zelí byl sledován ve třech cyklech a při čtyřech opakováních za kontrolovaných podmínek (2,5 % NaCl, 21 °C) při použití startovacích kultur (kontrola; *Leuconostoc mesenteroides* – 700 mil. cfu/ml; *Lactococcus lactis* ssp. *lactis* – 500 mil. cfu/ml; šťáva z předchozí fermentace). Každý cyklus trval 28 dní, během kterých byly sledovány mikrobiologické a chemické charakteristiky zelí. Denně byly sledovány množství mléčných bakterií, koncentrace kyseliny mléčné a pH média. Po skončení každého fermentačního cyklu následovalo stanovení organoleptických vlastností produktu, které pokračovalo dalších 6 měsíců s cílem zjistit dobu expirace. Z hlediska jakosti byly nejlepší výsledky dosaženy při použití startovací kultury *L. lactis* ssp. *lactis* při následné fermentaci flórou přirozeně se vyskytující v zelí (kontrolní varianta). Organoleptické vlastnosti produktu a jeho expirační doba byly při použití mléčné bakterie *L. mesenteroides* jako startovací kultury příznivější ve srovnání s použitím organolepticky nejlepší vyfermentované šťávy získané z předchozího fermentačního cyklu.

Klíčová slova: fermentace; mléčné bakterie; *L. lactis* ssp. *lactis*; *L. mesenteroides*; organoleptické vlastnosti; kyselé zelí

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