USE OF TWO PEDIOCOCCUS STRAINS ISOLATED FROM SOUR VEGETABLES AS STARTERS IN DRY SAUSAGE

E. Petäjä and E. Puolanne
Department of Food Technology, Meat Section, P.O. Box 27, FIN-00014 University of Helsinki, Helsinki, Finland

INTRODUCTION

When fermented meat products are prepared from whole meat by means of common starters, the pH values do not decrease as low as in fermented sausage, and the products do not ripen as firm as fermented sausages (Petäjä and Kuusela 1978). By using more effectively lactic acid-producing lactic acid bacteria more effective development of firmness may also be achieved in fermented whole-meat products. Some vegetables are known to have very low pH values (as low as 3.7); from these products effectively acid-producing lactic acid bacteria were isolated, and it was investigated if they could be applicable in fermented meat products. Their growth, their acid production with resultant pH decrease and effect on ripening and quality in fermented sausage were preliminarily studied. The purpose was to ensure that the strains isolated and selected for meat product studies work at least in dry sausage, which is a homogeneous fermented meat product.

MATERIAL AND METHODS

Isolation of the lactic acid bacterial strains: Lactic acid bacterial strains were isolated from the following fermented vegetable products: sour cabbage (HK), sour cabbage with marine algae (MLHK), sour carrot stripes (POHK), sour beans (HPA), and sour mixed vegetables (HSV). It was shown that the products contained 1 predominate lactic acid bacterial strain. All the 5 predominate strains were typed as belonging to the genus Pediococcus (cocci, tetrad grouping, catalase-negative, fermenting). Acid production of the 5 isolated strains was tested by growing them in APT broth, pH 5.6, for 2 days at 30°C. The pH values of the APT broths with various inocula were as follows: HK 3.95, MLHK 3.90, POHK 3.87, HPA 4.10 and HSV 4.06.
The MLHK and POHK strains were selected for sausage experiments as the strongest acid producers.
Preparation of sausages: Four experimental series of dry sausage, each 150 g, were prepared in a Moulinex blender. The sausages were prepared in a Moulinex-scale because of preliminary nature of the study. One series consisted of control sausages inoculated with RM 2000 starter (Pediococcus pentosaceus + Staphylococcus carnosus; Rudolf Müller & Co, Giessen, Germany), sausages inoculated with Pediococcus strain MLHK and Baktoferment 61 starter (Staphylococcus carnosus; Rudolf Müller & Co.) and sausages inoculated with Pediococcus strain POHK and Baktoferment 61 starter.
The experimental sausages were prepared according to the following formulation: beef 33.3%, pork 33.3%, pork fat 30.0%, NaCl 2.9%, glucose 0.3%, $NaNO_2$ 0.006% and KNO_3 0.012%. The strains MLHK and POHK were added as APT broth culture (10 ml/150 g sausage). RM 2000 was suspended in 10 ml sterile water prior to inoculation. Baktoferment 61 was suspended in APT broth cultures of MLHK and POHK strains prior to inoculation. The aim was to inoculate lactic acid bacteria at doses of 10^7 colony forming units (cfu)/g and staphylococci at 5 x 10^6 cfu/g into sausage. Raw materials and additives were placed in a Moulinex blender and coarse ground; thereafter the bacteria were inoculated and the mixing completed (particle size 3 mm in diameter). From each batch one 150-g sausage of was prepared, and the completed mixture stuffed into 45-mm collagen casing. One sausage was prepared for each ripening period; the ripening program of the sausages was as follows:

Ripening time	Temperature	Humidity	Smoking
1 day	22°C	96%	
2 - 7 days	20-21°C	96-90%	3 h/day
8 - 14 days	15°C	80%	

Determinations of the experimental sausages:
Sensory evaluation: The texture, aroma and flavour were evaluated after 3, 7, and 14 days of ripening, using a scoring system and descriptive method as follows:

- Texture (scores 6 - 0; 6 firm, 4 quite firm, 2 slightly firm, 1 - 0 soft)
- Aroma (scores 6 - 0; 6 excellent, 4 good, 2 odourless, 1 - 0 unpalatable)
- Flavour (scores 6 - 0; 6 exellent, 4 good, 2 moderate, 1 - 0 unpalatable)

The evaluation was performed in the laboratory by 2 technicians (the sample sausages were too small for panel evaluation), who determined the scores by consulting with each other.
pH value and titrated acid: The pH value was measured directly from the samples at 3 different sites (the mean was used as a result). Acid titration was conducted from the filtrate obtained from the 1:9 dilution, and the results were calculated as percentages of sausage.
Weight loss: Weigt loss was measured as percentages of the original weight.
Microbiological determinations: Each experimental series was studied microbiologically after manufacture (day 0) and after 3, 7 and 14 days of ripening. The following determinations were performed: Total plate count of aerobically growing bacteria (APT agar, BBL 10918, 4 days at 30°C), inoculated lactic acid bacteria (APT agar, BBL 10918, 4 days at 30°C), staphylococci (Baird-Parker agar, Labm 85 and X085, 2 days at 37°C), pseudomonads (GSP agar, Kielwein 1969, 4 days at 25°C), Brochothrix thermosphacta (STAA agar, Gardner 1966, 2 days at 22°C) and yeasts and molds (Rose-Bengal agar, Labm lab36 and X009, 2-4 days at 30°C).

RESULTS AND DISCUSSION

Sensory evaluation:
Texture: Sausages prepared with POHK pediococci were firmest after 7 and 14 days of ripening (Table 1). The mean texture value of MLHK sausages was also higher than the mean of RM 2000 sausages after 14 days of ripening; the texture score differences, however, were not significant.
Aroma: The aroma scores of the experimental sausage groups were not significantly different after 7 or 14 days of ripening (Table 1). The strongest aroma appeared in POHK sausages.
Flavour: The flavour scores of experimental sausage groups were not significantly different (Table 1). The strongest flavour appeared in POHK sausages and was slightly bitter.
pH value: At the beginning of ripening the pH values ranged 5.40 - 5.68 (means 5.5-5.6, Table 2). During 3 days of ripening the mean pH values decreased to <5; the lowest mean (4.80) was in the POHK sausage group. The pH of all the samples was <5 only in the POHK

sausage group. The pH values continued to decrease after 3 days of ripening in the MLHK and POHK sausage groups, the respective means being 4.70 and 4.63 after 7 days of ripening. During the following week the pH continued to decrease in the MLHK group.
It can be concluded that the Pediococcus strains from sour vegetable products decrease the pH to lower values than those attained with pediococci of the RM 2000 preparate. The lowest values, however were attained by vegetable pediococci until after 1-2 weeks of ripening.
Titrated acid content: The content of titrated acid was 0.6% at the beginning of ripening, increasing to 1% in RM 2000 sausages and to 1.3% in MLHK and POHK sausages during the first 7 days of ripening (Table 2). The titrated acid content continued to increase after 7 days of ripening, the means being 1.37% in MLHK sausages and 1.46% in POHK sausages after 14 days of ripening.
The titrated acid contents also showed that pediococci isolated from sour vegetables have stronger acid production capacity in dry sausage than do RM 2000 pediococci.
Weight loss: Experimental sausages dried to a weight loss level of 35% during 2 weeks of ripening; this weight loss is too high compared with sausages of normal caliber. Despite the high weight loss, respecting a_w value of 0.88, all inoculated lactic acid bacteria grew and survived.
Microbiological determinations: The count of inoculated lactic acid bacteria in RM 2000 sausage was very high, mean 8.0 log cfu/g just after inoculation, the respective counts being on the level of 7.0 log cfu/g in sour vegetable pediococcus sausages (Tables 3). The inoculated lactic acid bacteria formed the predominant part of the microbial flora of experimental sausages during the ripening period. The count of RM 2000 pediococci increased only 0.5 log units while the count of vegetable pediococci increased by 1 log unit or more during ripening. The increase in inoculated RM 2000 pediococci and MLHK vegetable pediococci occured during the first 3 days of ripening while the count of POHK vegetable pediococci increased during 2 weeks of ripening.
The mean count of staphylococci in RM 2000 sausages was 7.5 log cfu/g after inoculation, decreasing to 7.0 log cfu/g during 2 weeks of ripening. In sausages inoculated with sour vegetable pediococci the mean Staphylococcus count was 1 log unit less than in RM 2000 sausages after inoculation decreasing by 1 log unit or more during 2 weeks of ripening.
In the beginning of ripening the sausages contained pseudomonads (counts ranging 2.0 - 4.6 log cfu/g) and Brochothrix thermosphacta (counts ranging 2.0 - 5.5 log cfu/g). After 3 days of ripening the pseudomonad counts had decreased to <2.0 log cfu/g while the counts of Brochothrix thermosphacta decreased to <2.0 log cfu/g until during 7 days of ripening.
The experimental sausages contained yeasts the counts ranging from <2.0 - 4.7 log cfu/g. The yeasts appeared throughout 14-day ripening period, showing variable counts.

CONCLUSIONS

Two lactic acid bacterial strains isolated from sour vegetables can be applicable for use as starters in dry sausage on the following bases:

1. The two Pediococcus strains isolated from sour vegetables grew to >8.0 log cfu/g in dry sausage.
2. The sour vegetable Pediococcus strains formed more acid than starter pediococci from commercial preparate RM 2000, thus also decreasing the pH value. The pH of the sausages prepared with vegetable pediococci decreased to about 4.6.
3. The texture of sausages prepared with sour vegetable pediococci was firmer than the texture of RM 2000 sausages. Also the mean aroma and flavour scores were higher than those of RM 2000 sausages.

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TABLE 1. Texture, aroma and flavour (scores 6-0) of experimental sausages after 3, 7, and 14 days of ripening.

	3 days		7 days		14 days	
Sausage group	X	s	X	s	X	s
Texture						
1. RM 2000	2.5	0.6	4.0	1.4	5.3	1.0
2. MLHK	2.0	0.8	3.8	0.5	5.8	0.5
3. POHK	2.5	1.3	4.8	1.0	6.0	0.0
Aroma						
1. RM 2000	3.5	0.6	4.8	1.3	4.5	0.6
2. MLHK	3.8	1.0	4.3	1.3	5.0	0.8
3. POHK	3.5	0.6	4.5	0.6	5.3	1.0
Flavour						
1. RM 2000	3.0	1.0	4.3	1.0	4.8	0.5
2. MLHK	3.7	1.2	4.3	1.0	5.0	0.0
3. POHK	4.0	1.7	4.3	1.0	5.5	0.6

X = mean
s = standard deviation of mean
1) Means within the vertical line not followed by the same letter are significantly different ($p < 0.05$). If no letters are listed after the means, no differences are present among them.

TABLE 2. The pH values and titrated acid contents (%) of experimental sausages after 0, 3, 7, and 14 days of ripening.

	0 days		3 days		7 days		14 days	
Sausage group	X	s	X	s	X	s	X	s
pH value								
1. RM 2000	5.60	0.08	4.93	0.16	4.98[a]	0.07	4.99[a]	0.10
2. MLHK	5.51	0.09	4.99	0.20	4.70[ab]	0.25	4.58[ab]	0.08
3. POHK	5.56	0.04	4.80	0.03	4.63[b]	0.23	4.62[b]	0.20
Titrated acid (%)								
1. RM 2000	0.56	0.05	0.89	0.02	1.01	0.08	1.14[a]	0.14
2. MLHK	0.60	0.10	0.88	0.05	1.30	0.24	1.37[ab]	0.25
3. POHK	0.50	0.03	0.91	0.01	1.28	0.22	1.46[b]	0.19

TABLE 3. Total count of lactic acid bacteria (log colony forming units/g = log cfu/g) A-table) and count of inoculated lactic acid bacteria (log cfu/g; B-table) in experimental sausages after 0, 3, 7, and 14 days of ripening.

	0 days		3 days		7 days		14 days	
Sausage group	X	s	X	s	X	s	x	s
A-table								
1. RM 2000	1)8.0	0.2	8.6	0.4	8.3	0.3	8.4	0.1
2. MLHK	7.7	0.3	8.6	0.3	8.7	0.3	8.3	0.7
3. POHK	7.1	0.7	7.9	1.1	8.1	0.5	8.2	0.6
B-table								
1. RM 2000	8.0[a]	0.2	8.6	0.4	8.3	0.3	8.4	0.1
2. MLHK	7.3[ab]	0.6	8.3	0.6	8.5	0.3	8.0	0.5
3. POHK	6.9[b]	0.8	7.7	0.9	8.0	0.4	8.5	0.7