APPLICABILITY OF LACTIC ACID BACTERIA FOR USE AS STARTERS IN FERMENTED FISH

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INTRODUCTION

Different types of naturally fermented fish products are known in different areas of the world, but information on the whole fish products fermented by starter lactic acid bacteria (LAB) have not been reported before Morzel et al. (1997) who have just published their research concerning salmon fermentation by LAB. They report a firmer texture and lighter colour of fermented (pH ~5,5) than fresh salmon. The purpose of this investigation was to study what type of product can be brought about by fermenting fish with LAB; is it possible to ferment fish with LAB in the same way as the meat of mammals.

MATERIAL AND METHODS

Preparation of fermented fish fillets: Five series of fermented rainbow trout fillets were prepared with the following inocula:

1. Control fillet without any inoculum
2. *Pediococcus pentosaceus* strain "strong acid producer" (SPX-starter; Chr. Hansen A/S, Hörsholm, Denmark) and *Staphylococcus carnosus* strain (Pökelferment 77-starter; Rudolf Müller & Co., Giessen, Germany)
3. *Lactobacillus pentosus* strain "rapid acid producer" (SL-starter; Chr. Hansen A/S, Hörsholm, Denmark) and S. *carnosus*
4. *Pediococcus* strain MLHK (from sour vegetables; Petäjä and Puolanne 1997) and *S. carnosus*
5. *Pediococcus* strain POHK (from sour vegetables; Petäjä and Puolanne 1997) and *S. carnosus*
6. *Pediococcus* strain POHK

Staphylococci (st.) were inoculated into fillets in order to eliminate the H_2O_2 formed by LAB and to prevent the oxidizing defects of H_2O_2. The experimental fillets were cured by injecting brine in amounts corresponding to 5% of the weight of the fish fillet. The injected brine contained 20% NaCl, 6% or 18% glucose, 0.5% ascorbic acid and 0.625% KNO_3. Two preliminary experimental series were prepared with the 0,6% glucose in the brine (0.3 % in the product). As the pH of the fillets was not reduced enough, the glucose concentration of the brine was increased to 18% (0.9% in the product) in the main three experimental series. LAB (used as the APT broth culture 10^7 cfu/g fish) and st. (commercial preparate $5x10^6$ cfu/g fish)were inoculated into the fillets in addition to the brine. Curing was completed by dry-salting fillets with 2% coarse salt for 1 day at 6°C and thereafter hung in a fermenting chamber. Fermented fillets were packed in a vacuum and ripened at 7-8 °C for 1 week and thereafter stored at 4 °C (to test self-life at 28 days).

Fermenting time	Temperature	Humidity Smoking
1 day	22 °C	96 %
2 - 7 days	21 - 20 °C	96 - 90 % 3 h / day
8 - 16	15 °C	80 %

Determinations on the experimental fillets:

pH value and titrated acid: The pH value was measured from a 1:9 dilution (0.9% NaCl solution). Acid titrations (percentages of the fillets) were conducted on the filtrates obtained from the dilution. The measurements were determined from the raw fish material (0 d.) and after 2, 3, 5 and 7 days of fermenting

Weight loss and a_w-value: Weight losses were measured as percentages of the original weight and a_w-values with Luft-a_w-measurer (Luft, G. Luft Mess und Regelteknik Gmbh, Germany) after 2, 3, 5 and 7 days of fermenting.

Microbiological determinations: Each experimental series was examined microbiologically after manufacture (0 d.) and after 2, 3, 5 and 7 days of fermenting. The following determinations were performed: total plate count of aerobically growing bacteria (APT agar/pH 7.0, BBL 10918, 4 days at 30°C), inoculated lactic acid bacteria (APT agar/pH 5.6, 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), yeasts and moulds (Rose-Bengal agar, Labm lab36 and X009, 2-4 days at 30°C) and sulphite-reducing clostridia (SPS agar, BBL, 2 days at 37°C anaerobically).

Sensory evaluation: The evaluation was performed by a 6-person-panel familiar with sensory evaluation of foodstuffs; desired quality was established with training evaluations. The texture, aroma and flavour were evaluated after 3, 5 and 7 days of ripening using a scoring system and descriptive method as follows: Ripeness (texture): scores 6 - 0; 6 expected dry, 4 less dry, 2 slightly soft, 0 soft; Aroma: scores 6 - 0: 6 excellent, 4 good, 2 odourless, 0 unpalatable; Flavour: scores 6 - 0; 6 excellent, 4 good, 2 moderate, 0 unpalatable

Statistical methods: The results of the main three series were subjected to statistical tests (analysis of variance/Stat Graphics Win 32).

RESULTS AND DISCUSSION

pH value: In the control group the pH value decreased from 6.6 to 6.0 during 7 days of fermenting (Table 1). In the inoculated fish fillets, excluding POHK + st. samples the pH decreased remarkably (by at least 1.3 unit) during 7 days of fermenting. The pH of fish fillets inoculated with SL-lactobacilli decreased most rapidly, pH being 0.2 pH-unit lower than with other inocula after 2 and 3 days of fermenting. On the other hand MLHK-pediococci reduced pH to lower values than other inocula, the largest decreases being between 5 and 7 days of fermenting (pH 4.9 after 7 days of fermenting). The pH of all inoculated fish fillet groups was significantly lower than the control group after 5 days of fermenting. This was also the case after 7 days of fermenting excluding the POHK + st. group.

It can be concluded that by using lactic acid bacterial strains from commercial starter products or *Pediococcus* strains from sour vegetables the pH of fish may be lowered to almost the same values as in fermented meat products. To achieve this decrease the content of glucose must be three times higher than the content used in fermented meat products. Higher amounts of glucose are needed because the pH of fish is about one pH unit higher than the pH of meat and because the strong buffering capacity of fish.

Titrated acid content: During the 7 days of fermenting the content of titrated acid of experimental fillets increased from initial 0,7% to 1.4 - 1.8%, excluding the POHK + st. group. The acid content of the control fish fillet group remained at 1%. There were no significant differences between the sample groups at any stage in the experiment.

Weight loss and a_w-value: The mean weight losses: 5.7% (2 days fermented), 11.3% (3 d.), 21.3% (5 d.) and 30.2% (7 d.). a_w-values of the fillets decreased as follows: 0.980 after preparing (0 day), 0.960 after 2 days of fermenting, 0.952 (3 d.), 0.940 (5 d.) and 0.927 (7 d.).

Microbiological determinations: The means of total count of bacteria corresponded to the count of inoculated LAB ranged 6.9-7.7 log cfu/g after inoculation (Table 2). The count of inoculated SL-lactobacilli increased most during the first two days of ripening (to 8,3 log cfu/g), thereafter increasing only slightly. The mean counts of other inoculated fish fillet groups increased to over 8.5 log cfu/g during 5 days of ripening. The inoculated LAB formed the predominant part of the microbial flora of the experimental fillets during the ripening period. This was confirmed by microscoping 3 predominant type of colonies. The total bacterial count of control fillet group increased from 3.7 log cfu/g to over 7.0 log cfu/g during the first 3 ripening days and remained at that level.

The mean count of staphylococci in fillets after inoculation was 6.5 log cfu/g increasing to over 7.0 log cfu/g during the first 3 days of ripening remaining at that level. Only in the SL + st. group the count of staphylococci remained on the same level as it was after inoculation. In the control and POHK fillet groups the mean staphylococcus count was 4.4 log cfu/g at the beginning of fermenting increasing to over 7.0 log cfu/g over 5 days of fermenting in control group and over 3 days in POHK group. This strong increase in staphylococcus counts in the fillets which were not inoculated with staphylococci was unexpected.

The mean pseudomonas count of raw material was 4.3 log cfu/g. After the first two days of fermenting the fillets contained pseudomonads 2.8 - 4.3 log cfu/g. In the control fillet group pseudomonads appeared during the whole fermenting period over 2.0 log cfu/g excluding one sample. In the inoculated groups the appearance of pseudomonads over 2.0 log cfu/g was more accidental after 2 days of ripening. The mean yeast counts of raw material was 4.2 log cfu/g. Thereafter, the yeast counts over 2.0 log cfu/g appeared only sporadically during the whole ripening period. The fillets did not contain sulphite reducing clostridia over 1 log cfu/g during fermentation.

Shelf-life: When the fillets of the experimental series were stored after fermentation and ripening (7-8°C) for 14 days at 4°C., they proved sensorically desirable, pH values and the counts of LAB had remained at the same level as at the end of fermentation. The colour and texture were even balanced throughout the whole fillet. When the fillets were tasted after a storage period of 3 months the products with a low pH (<5.3) were still acceptable.

Sensory evaluation:

Texture: There was no difference between the texture values of fish fillet groups after 3 days of fermenting. After 5 days of fermenting all the inoculated fillet groups, excluding the POHK + staf. group, were significantly firmer than the control group. After 7 days of fermenting all inoculated groups were firmer than the control group.

Aroma: The aroma values were not significantly different after 3, 5 and 7 days of fermenting.

Flavour: The flavour values of experimental fish fillet groups were not significantly different after 3 days of fermenting. After 5 days of fermenting all the inoculated fillet groups excluding POHK + staf. and POHK groups were evaluated as significantly better than the control group. After 7 days of fermenting there were again no differences between fillet groups.

Table 1. The pH value of experimental fish products after 0, 2, 3, 5 and 7 days of fermenting

Fillet group	0 day		2 days		3 d		5d		7 d	
	x	s	x	s	x	s	x	s	x	s
Control	6,6	0,1	6,3	0,2	6,1	0,0	6,1a	0,1	6,0a	0,0
SPX + st	6,6	0,1	6,2	0,4	5,9	0,3	5,2b	0,3	5,2b	0,2
SL + st	6,6	0,1	6,0	0,1	5,6	0,4	5,0b	0,1	5,2b	0,3
MLHK+st	6,6	0,1	6,2	0,5	5,9	0,0	5,2b	0,2	4,9b	0,5
POHK+st	6,6	0,1	6,2	0,2	5,8	0,3	5,7b	0,3	6,0a	0,4
POHK	6,6	0,1	6,3	0,3	6,1	0,5	5,3b	0,2	5,3b	0,6

Table 2. Count of lactic acid bacteria on APT (pH5,6) -agar (cfu/g) after 0, 2, 3, 5 and 7 days of fermenting

Fillet group	0day		2 days		3 d		5 d		7 d	
	x	s	x	s	x	s	x	s	x	s
Control	3,7a	0,6	5,8a	0,3	7,3a	0,9	7,5	1,5	7,4a	0,7
SPX + st	7,7b	0,5	7,8b	0,7	8,1b	0,4	8,9	0,3	8,7b	0,2
SL + st	7,4c	0,2	8,3b	0,3	8,0a	0,1	8,3	0,3	8,5b	0,1
MLHK+st	6,9c	0,3	7,6b	1,1	8,3b	0,3	8,9	0,4	8,8b	0,2
POHK+st	7,2bc	0,5	7,7b	0,3	8,0b	0,4	8,6	0,9	8,2b	0,3
POHK	7,0bc	0,5	7,3b	0,2	8,1b	0,2	9,1	0,8	8,7b	0,2

x= mean, s= standard deviation of mean

Means within the vertical line not followed by the same letter are significantly different ($p<0,05$). If no letters are listed ; no differences.

CONCLUSIONS

1. Glucose must be added at almost 1% to fish to reduce its pH to between 4.9 and 5.3. These levels correspond to safe pH values for fermented meat products. Lactic acid bacteria reduced the pH in fish only to a level of >5.6 when 0.3% glucose was used.
2. The inoculated lactic acid bacterial strains grew to >8.5 log cfu/g.
3. The colour of the inoculated products turned bright pink, the texture was dryer than the control fillets and the flavour sourish. However, this sour flavour was accepted to the panel members.
4. The inoculated products could be safely kept for at least 4 weeks.

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