

Lactic acid fermentation of fish offal and chicken by-product with different starter cultures

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Lactic acid fermentation was evaluated as a method to preserve fish and chicken by-products. Herring (*Clupea harengus*) by-products (viscera and heads) and chicken by-products (heads, viscera, feathers, feet and discarded whole chickens) were minced, mixed with 5% dextrose and inoculated with 10^8 colony forming units (cfu)/g of four different lactic acid bacteria cultures. The by-product was fermented at 25°C and evaluated for pH, % produced lactic acid, redox potential and odour during four weeks' storage. In herring offal, pH decreased from 6.8 to 4.2 in one week and stabilized at about 4.3. In the same time, 2.0% to 3.2% lactic acid was produced and concentrations stabilized from 2.5% to 4.0%. In chicken offal, pH decreased to a stable level of 4.4, and 3.2% lactic acid was produced after one week of fermentation. A negative and stable redox potential was achieved after one week of fermentation in both herring and chicken offal.

Key words: animal waste, silage, lactic acid bacteria, redox potential, mink feed

Introduction

Animal by-products are obtained in feed plants (pet food and fur animal feed) in which the raw material is processed in different ways, depending on the origin and destination of the feed. The most common processing methods are heating, drying, chilling and freezing.

The increasing cost of energy has aroused interest in developing alternative storage methods. Promising results have been obtained in

preserving fish and slaughter-house by-products with lactic acid bacteria (LAB) (Kangas and Kangas 1983, Lindgren and Pleje 1983, Hassan and Heath 1986, Tibbets et al. 1987, Skrede and Nes 1988, Russel et al. 1992, Urlings 1992). Fermented silage is a product of a controlled fermentation process in which production of lactic acid (LA) results in a pH below 4.5. Owing to the lack of fermentable carbohydrates and a natural LAB flora, animal by-products cannot be subjected to lactic acid fermentation without the addition of a substrate. The methods used

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are based on the addition of LAB and fermentable carbohydrates to minced animal by-products.

The preservative action of LAB in animal by-products is caused by low pH, a substantial amount of undissociated organic molecules, the buffering capacity of the raw material, hydrogen peroxide production, competition with other bacteria for nutrients, production of antibiotics and bacteriocines (bacteriocines are most often bacteriostatic to closely related species) and decreased redox potential (Raccach and Baker 1978, Smith and Palumbo 1981). The lowering in pH and the effect of undissociated LA inhibit growth of bacteria such as *Staphylococcus* (Bartholomew and Blumer 1980), *Escherichia coli* (Tramer 1966), *Serratia*, *Enterobacter*, *Citrobacter*, *Achromobacter* and *Pseudomonas* (Dubois et al. 1979).

Bacteria strains commonly used as starter cultures are *Lactobacillus plantarum* (Kangas and Kangas 1983, Lindgren and Pleje 1983, Skrede and Nes 1988, Urlings 1992), *Pediococcus acidilactici* (Lindgren and Pleje 1983), *Lactobacillus acidophilus* (Tibbets et al. 1987) and some not fully characterized commercial cultures (Pelzyme®, Lassén et al. 1990a, b, PSI®, Russel et al. 1992, Stabisol®, Partanen et al. 1992). With a suitable starter culture, a mixture of fish or slaughter by-products and carbohydrates may reach a pH of 4.4 to 5.0 in 24 to 48 h (Lindgren and Pleje 1983). Silage of this kind has been used as a component of feed for fur-bearing animals (Skrede and Nes 1988, Lassén et al. 1990a, b, Urlings 1992) and pigs (Tibbets et al. 1987, Partanen et al. 1992) with varying results.

LAB fermentation of animal by-products may be worth developing as an alternative storage method, due to its economic advantages and its potential to kill pathogens (Wooley et al. 1981). To produce a quality feed product, the conditions for production from various raw materials have to be systematically evaluated, and efficient methods for quality control of the product developed. This paper examines the conditions for fermentating herring and chicken offal.

Material and methods

Fish offal was fermented at the Royal Veterinary and Agricultural University, Denmark and chicken offal at FinnEwos Agri, Kariniemi, Finland.

Fish offal (viscera and heads) from herring (*Clupea harengus*) caught in the North Sea in autumn was obtained from Gilleleje Processing Plant, Gilleleje, Denmark. The offal was minced coarsely once through a 10-mm plate and deep-frozen within 6 h. The offal was kept at -20°C until 12 h before it was to be used. All fermentations were conducted using thawed herring offal, that had been mixed thoroughly in a Vari-mixer bakery machine and incubated at 25°C . Fermentation was conducted with commercial starter cultures containing *Lactobacillus plantarum* (L.p), *Lactobacillus plantarum*:*Pediococcus pentosaceus* (L.p:P.p 1:2), *Lactobacillus plantarum*:*Pediococcus acidilactici* (L.p:P.a 1:2) (Chr. Hansen's Lab. A/S, Hørsholm, Denmark), and the commercial Pelzyme® (Cultor Ltd., Feed Division, Helsinki, Finland). The herring offal was fermented for one week in 3-kg portions with 5% (w/w) dextrose added and an initial inoculum of 10^8 colony forming units (cfu)/g in 5 l plastic containers equipped with a water airtight. After fermentation the material was stored at 25°C for four weeks.

Chicken by-products were obtained from 39 day-old chickens and processed at the chicken slaughterhouse as follows. Immediately after slaughter of the chickens by-products (heads, viscera, feet and discarded whole chickens) were minced through a 10-mm plate, homogenized through a 6-mm plate and mixed with 12% (w/w) extruded wheatmeal and feathers (2:1) according to the FinnEwos Agri method®; 5% dextrose was then added. Finally, the starter culture was added when the material to be fermented had reached a temperature of 25°C . The same starter cultures were used as for fermentation of herring offal, with an initial inoculum of 10^8 cfu/g. All fermentations were conducted in triplicate at 25°C in the same types of plastic container as

used for herring offal. The fermented material was stored at 25°C for four weeks.

Treatment effects were evaluated by monitoring the pH values, LA production and changes in redox potential during four weeks of storage as previously described (Lassén 1995a). All three samples from each batch were analysed weekly for pH and LA production, and since earlier experiments (Lassén 1995a) had yielded very stable and reproducible results within treatments and time, only one sample per batch was measured for redox potential.

Proximate analysis of herring and chicken offal before and after fermentation was conducted according to standard methods. The dry matter content was analysed by drying 4 g samples in a forced draught oven at 60°C for 18 h. Nitrogen was determined by the Kjeldahl procedure and protein calculated as N x 6.25. Fat was hydrolysed with HCl, extracted with petroleum ether and analysed by the Soxtec method. Soluble and hydrolysable hexose carbohydrates (dextrose) were analysed according to Jacobsen (1981).

Statistical analyses were performed according to the GLM procedure in SAS (SAS 1985). Changes in pH and LA production were evaluated separately for the fermentation of herring offal and chicken waste (1) and for the materials together (2) according to the following split-plot design model:

$$Y_{ijk} = \mu + a_i + b_j + c(i) + (ab)_{ij} + \epsilon_{ijk}$$

where,

Y_{ijk} = the ijk th observation,

μ = general mean,

a_i = fixed effect of starter culture,

i = 1-4 (1) or material, i = 1 or 2 (2),

b_j = fixed effect of storage time, j = 0, 1, 2, 3 and 4 weeks (1 and 2),

$(ab)_{ij}$ = interaction effect between starter culture,

i = 1-4 (1), or material i = 1 or 2 (2) and storage time, 0-4 weeks (1 and 2),

$c(i)$ = random sample within starter culture (1) or material (2),

ϵ_{ijk} = random error.

Table 1. LA-production and changes in pH in herring offal fermented with different starter cultures, 10^8 cfu/g, and 5% dextrose, and chicken offal fermented with 5% dextrose and 12% extruded wheatmeal:feathers (2:1) and 10^8 cfu/g at 25°C.

Storage time, weeks	pH		% LA	
	Herring	Chicken	Herring	Chicken
0	6.8	6.2	0.0	0.0
1	4.3	4.5	2.3 ^a	3.2 ^b
2	4.1	4.3	2.8 ^a	4.1 ^b
3	4.2	4.4	2.8 ^a	4.1 ^b
4	4.4	4.4	2.4 ^a	4.0 ^b

a b Values differ significantly ($P < 0.01$).

LA= Lactic acid

cfu = colony forming units

Treatment effect were tested against the silo effect, thus using silos within starter culture (1) or material (2) as an error term.

Results

Fermentation of herring offal with 5% dextrose at 25°C resulted in a drop in pH from 6.8 to 4.3 (L.p) and from 6.8 to 4.0 (Pelzyme®), in one week; pH stabilized at between 3.9 and 4.5 after four weeks (Table 1). Rapid LA production was observed during the first week of fermentation, which stabilized at between 2% and 4% after four weeks of storage (Fig. 1a). The effect on redox potential showed a similar pattern, Pelzyme® having the most negative redox potential after four weeks (Fig. 1b).

Chicken offal was fermented successfully. Fermentation with 5% dextrose and 12% extruded wheatmeal:feathers (2:1) resulted in rapid LA production (2.8% to 3.2%), and a pH drop from 6.2 to 4.4 in one week (Fig. 2a), followed by a negative redox potential (Fig. 2b). Pelzyme® resulted in a highly significant pH decrease

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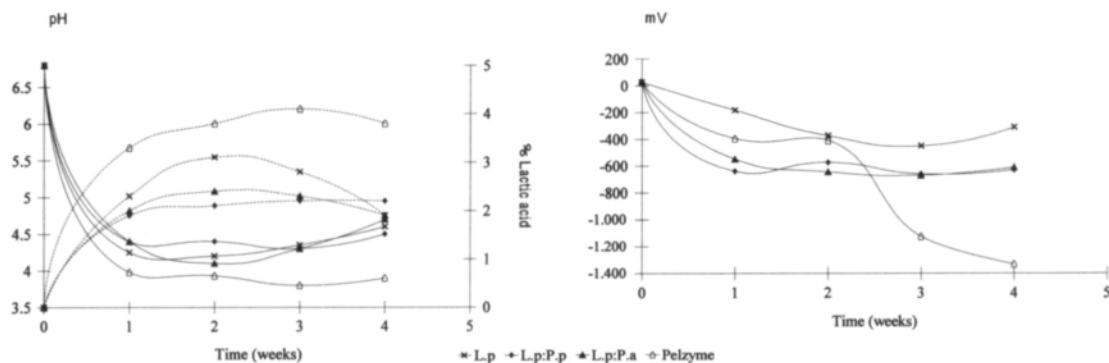


Fig. 1. Lactic acid fermentation of herring offal with different LAB cultures, 10^8 colony forming units/g and 5% dextrose at 25°C. a) — Changes in pH, — % LA. b) — Changes in redox potential.

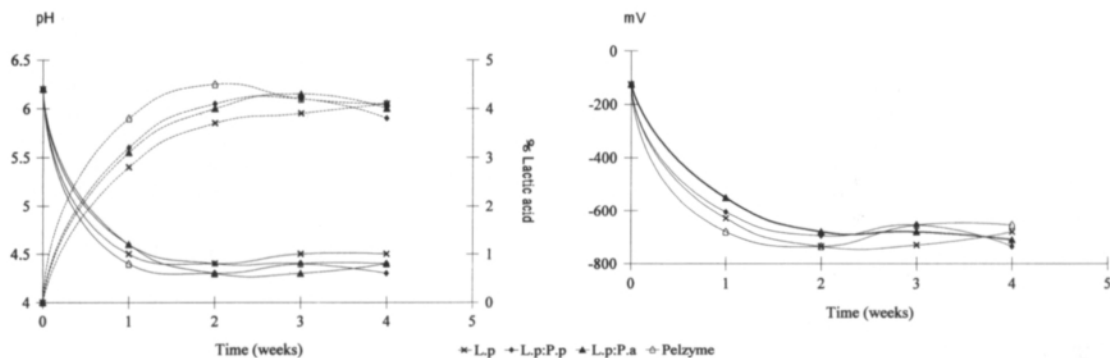


Fig. 2. Fermentation of chicken offal with different LAB cultures, 10^8 colony forming units/g, 5% dextrose and 12% extruded wheatmeal:feathers (2:1) at 25°C. a) — Changes in pH, — % LA. b) — Changes in redox potential.

($P < 0.01$) during the first week of fermentation, followed by stabilization at about pH 3.9. The other cultures tested were initially slower than Pelzyme®, but still showed a highly significant LA production and pH decrease after two weeks' storage. The difference in pH and LA production between the LAB cultures tested and Pelzyme® was significant ($P < 0.01$) when herring offal was fermented, but no significance was noted between LAB cultures when chicken offal was used. Eventually a highly significant ($p < 0.01$) difference in LA production between different materials was found for all cultures used in the present study; the pH decrease was, however, nonsignificant (Table 1).

The freshly minced herring had the texture and appearance of coarsely minced fish, where-

as the fermented offal was liquefied and had a clear layer of oil on the top. Chicken offal did not liquefy as much as herring offal during storage. In fermented herring and chicken offal the protein content in dry matter basis increased after fermentation for one week. The offal did not contain fermentable carbohydrates before addition of 5% dextrose (herring offal) and 12% extruded wheatmeal:feathers (2:1) and 5% dextrose (chicken offal), and only low concentrations (0.6% and 3.2%) remained after fermentation (Table 2).

Conversion of dextrose/carbohydrates to LA was more effective in chicken offal than in herring offal (Fig. 3). In fermented herring offal, the decrease in dextrose did not, however, correspond with LA production, and only 40% to

Table 2. Proximate composition of herring and chicken offal before and after fermentation for one week at 25°C.

Offal	DM %	Fat % of DM	Protein % of DM	Sugar % of DM	Starch % of DM
Herring ^a	29.3	38.6	35.4	14.6	0.0
Fermented herring	22.2	26.7	40.8	4.4	0.0
Chicken ^b	39.6	22.2	36.1	14.9	12.1
Fermented chicken	37.9	26.7	41.0	3.2	2.4

^a Herring offal, 5% dextrose, and 10⁸cfu/g *Lactobacillus plantarum*

^b Chicken offal, 5% dextrose, 12% extruded wheatmeal:feathers (2:1), and 10⁸cfu/g *Lactobacillus plantarum*
cfu = colony forming units

45% of the dextrose was metabolized to LA with the *Lactobacillus plantarum*-based cultures tested. Mainly in herring offal, when Pelzyme[®] was used as starter culture, the conversion of dextrose to LA was 80% complete, and consequently more acid was produced (Fig. 1a).

Discussion

Fast fermentation of herring offal with a rapid drop in pH to 4.4 or less resulted in a less putrefactive odour and a more stable silage than in silages produced at slower pH declines. In the literature, a preserving pH has been reported to occur after 24–72 h (Mackie et al. 1971, Raa 1980, Lindgren and Pleje 1983, Hassan and Heath 1986, Lassén et al. 1990a, b). Usually, a higher amount of lactic acid has to be produced in fish silage to yield a properly fermented product than in grass silage and silage from slaughter-house wastes. Lindgren and Pleje (1983) found that the LA content giving a pH below 4.4 was above 4.0% in fish silage, about 3.0% in slaughter-house wastes and below 2.0% in grass silage. Hassan and Heath (1986) reported that 4.4% LA was needed to obtain a pH of 4.2 in fish silage fermented with 5% lactose as the carbohydrate source. In the present study with its different starter cultures, 2.7% (L.p), 2.3% (L.p:P.p), 2.5% (L.p:P.a), and 2.5% (Pelzyme[®])

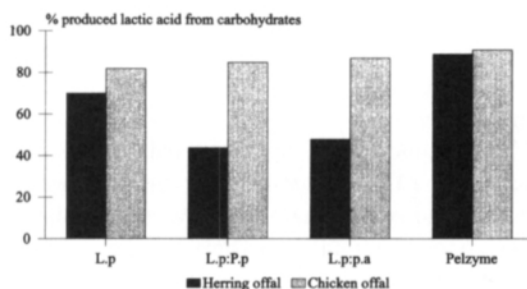


Fig. 3. LA production from 5% dextrose for different LAB cultures (10⁸ colony forming units/g) in silage made from herring and from 5% dextrose and 12% extruded wheatmeal:feathers (2:1) in silage made from chicken offal at 25°C.

LA were needed to obtain a pH below 4.2, and 1.8% LA in fish silage resulted in a pH below 4.4, all concentrations being below the concentrations reported in the literature. Successful fermentation of chicken offal was characterized by a higher initial lactic acid production to obtain a pH below 4.5; thus owing to its higher bone content, chicken offal is likely to have a higher buffering capacity than herring offal. After three weeks' storage a minor drop in LA content was observed, possibly due to the hydrolysis of bone material, neutralizing the acid. This may also be followed by an accumulation of biogenic amines (Lassén 1995b), which also neutralized the material. A high LA concentration at pH 4.3 gives a high concentration of undissociated LA molecules; these provide the antimicrobial effect but

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also inhibit further growth of LAB (Baird-Parker 1980).

Measurements of redox potential may be used to evaluate the growth conditions of anaerobic bacteria (Wolstrup 1972) and to determine the oxygen content of biological material (Harrison 1971). Lactobacilli are generally anaerobic to microaerophilic, demanding negative redox potentials, but certain strains grow aerobically and may lower the pH even under aerobic conditions (Kandler and Weiss 1986). Redox potential is always positive in the muscle and negative in the viscera of freshly caught herring (Huss and Larsen 1979). The redox potential in warm-blooded animals attains a negative value shortly after the animals have been slaughtered and remains negative until aerobic deterioration sets in (Barnes and Ingram 1955, Wirth and Leistner 1970). Rapid establishment of the LAB is important for LA production and the decrease in pH and redox potential. If the material reaches a low redox potential in a short time, growth of anaerobic bacteria, usually of the genera *Alteromonas*, is inhibited. These bacteria are implicated in the spoilage of North Sea fish (Shewan 1977). Other bacteria of the genus *Salmonella* are also known to be inhibited by a low redox potential (Chung and Goepfert 1970). Here, a low redox potential (-330mV to -680 mV) was achieved after one week of fermentation, the level being somewhat lower for silage of herring offal than for silage of chicken offal. The results of this and an earlier study (Lassén 1995a) clearly show that appropriate fermentation leads to a low and stable redox potential, whereas inappropriate fermentation leads to an increasing redox potential and pH as a result of decreased LA concentration.

The more efficient use of dextrose as a car-

bohydrate source when chicken offal was fermented might be explained by the lower number of actively competing bacteria. Bacteria in chicken have optimal growth conditions at about 30°C to 37°C, but fish bacteria are more active at lower temperatures (15°-25°C) and compete with LAB for free dextrose (Jay 1986). The changes recorded in chemical content after fermentation were probably due to separation processes in the material, and were therefore considered not to reflect true changes in chemical composition as an effect of the fermentation process. The decreased fat content in dry matter after fermentation might be explained by the separation process, in which the fat fraction is situated on top of the silo and the sample is taken from the bottom of the silo.

The result of this study suggest that animal by-products can be fermented with 5% dextrose and 10⁸ cfu LAB/g at 25°C, and that fermentation is thus a viable alternative to current offal handling practices. Successful fermentation depends, however, on the hygienic quality of the raw material and good hygiene at the processing plant. To improve the process, there is still a need for development of better starter cultures and methods for determining product quality.

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SELOSTUS

Kala- ja kanajätteiden maitohappofermentaatio erilaisilla mikrobiviljelmillä

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Tutkimuksessa selvitettiin, soveltuuko maitohappofermentaatio kala- ja kanajätteiden säilömismenetelmäksi. Sillin (*Clupea harengus*) jätteet (sisälmykset ja päät) ja kanajätteet (päät, sisälmykset, höyhenet, jalat ja hylätyt kokonaiset kanat) hienonnettiin, sekoitettiin 5 %:een dekstroosiin ja siirrostettiin neljään erilaiseen 10^8 cfu/g maitohappobakteeriviljelmään. Tätä sivutuotetta fermentoitiin 25 °C lämpötilassa. pH, maitohappopitoisuus %, redox-potenti-

aali ja haju tutkittiin neljän viikon varastointiaikana.

Sillijätteen pH laski 6,8:sta 4,2:een viikossa ja vakiintui 4,3:een. Samanaikaisesti maitohappopitoisuus oli 2,0 % – 3,2 % ja pitoisuudet vakiintuivat 2,5 %:sta 4,0 %:iin. Kanajätteen pH laski ja vakiintui 4,4:ään, ja maitohappopitoisuus oli 3,2 % viikon fermentaation jälkeen. Sekä silli- että kanajätteellä oli negatiivinen ja vakaa redox-potentiaali viikon fermentaation jälkeen.