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PII: S0308-8146(96)00291-9

*Food Chemistry*, Vol. 60, No. 1, pp. 13–18, 1997

0308-8146/97 $17.00+0.00

# Biotransformation of fish waste into a stable feed ingredient

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(Received 15 January 1996; revised version received 22 April 1996; accepted 22 April 1996)

Chopped pilchard wastes, including viscera, heads and tails, were mixed with 25% molasses and inoculated with a starter culture composed of *Saccharomyces* sp. and *Lactobacillus plantarum*. The silage was incubated at 22°C. Changes in nutritional quality and biochemical properties (pH, dry matter, ash, total and volatile nitrogen, lipids and trimethylamine) were monitored during a fermentation period of 15 days. Microbiological determinations were also carried out, including standard plate count, coliforms, *Clostridium*, lipolytic and proteolytic microorganisms. Results indicated that the pH decreased considerably and remained constant at 4.2 and 4.5 in the two trials. The total nitrogen decreased while the non-protein nitrogen and total volatile nitrogen increased significantly. Almost the same pattern in the two trials was observed. The trimethylamine decreased or remained constant at low levels depending on the initial value. The microbiological characteristics showed a rapid decrease of coliform and *Clostridium* counts to reach a low level after 5–7 days. Lipolytic and proteolytic microoganisms decreased notably during the fermentation and reached a minimum after 8 days and 5 days, respectively, in the two trials. © 1997 Elsevier Science Ltd

## INTRODUCTION

Large amounts of fish waste and deteriorated whole fish are discarded daily in the canning industry and fish markets in Morocco. These amounts could be recycled as a potential source of high-protein feedstuff in animal feeds (livestock, poultry, fish). The conversion of fish waste into fish meal by drying would be a high-cost, complicated process. The fermentation of fish waste is more suitable and convenient for small industries and/or the farmer.

Numerous reports have been made about fish silage production and its use in animal feed (Raa *et al.*, 1983; Jackson *et al.*, 1984*a*,*b*; Haaland & Njaa, 1989, 1990; Espe *et al.*, 1989, 1992; Espe & Haaland, 1992). So far, fish silage is still not known to Moroccan industry and farmers, despite the scarcity of animal feed ingredients.

Fish waste can be ensiled by (1) biological fermentation using lactic acid bacteria which exist naturally in the raw material or are introduced as starter cultures, or (2) chemical acidification using inorganic and/or organic acids (Raa *et al.*, 1983).

The use of combined starter cultures of yeasts and lactic acid bacteria to ferment fish waste has not been studied in detail. Fermentation was studied by Faid *et al.* (1994) as a biological process to preserve fish waste through mixed fermentation (alcoholic/lactic) and also to remove the fish odour.

In the present study, fish waste was processed by biological fermentation using a combined starter culture of *Saccharomyces* sp. and *Lactobacillus plantarum*. The changes in chemical and properties and the microbial profiles were monitored for 15 days.

## MATERIALS AND METHODS

### Fish waste/molasses preparation

Fish waste of the species *Sardina pilchardus* were obtained from the fish market in Rabat. Waste was chopped and mixed with cane molasses in the proportions 1:4; 2 kg of the mixture was then introduced into a

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5 litre plastic container. The starter culture was grown on molasses supplied with yeast extract (0.2%) for *Saccharomyces* sp. and on molasses supplied with yeast extract (0.3%) and peptone of casein (1%) for *Lactobacillus plantarum*. The culture was incubated for 48 h at 30°C. The mixture was inoculated with 5% of the starter culture and incubated at 22 ± 2°C.

### Chemical analyses

The pH was determined using a pH-meter (Crison MicropH 2000). Dry matter was determined by oven-drying a weighed amount of the product at 105°C until constant weight. Ash was determined after heating in a furnace at 550°C for 6 h. Fat content was determined on the dry matter by the soxhlet method; hexane was used as solvent. Total nitrogen (TN) was determined by the Kjeldhal method described by the APHA (1989). The non-protein nitrogen was also determined by the Kjeldahl method on the filtrate after precipitating the protein with 10% trichloroacetic acid solution. The total volatile nitrogen (TVN) content was measured by the method described by Conway (1947). Trimethylamine (TMA) content was determined according to the method described by Murray & Gibson (1972).

Microbiological analyses for coliform evaluation were determined by pouring plating dilutions from $10^{-1}$ to $10^{-4}$ on MacConkey glucose agar. In the basal formula described by Leininger (1976), lactose was substituted by glucose. The plates were incubated at 37°C. Proteolytic and lipolytic microorganisms were determined according to the methods described by Lee (1976) and Alford (1976), respectively. *Clostridium* counts were determined by the method described by Faid *et al.* (1990).

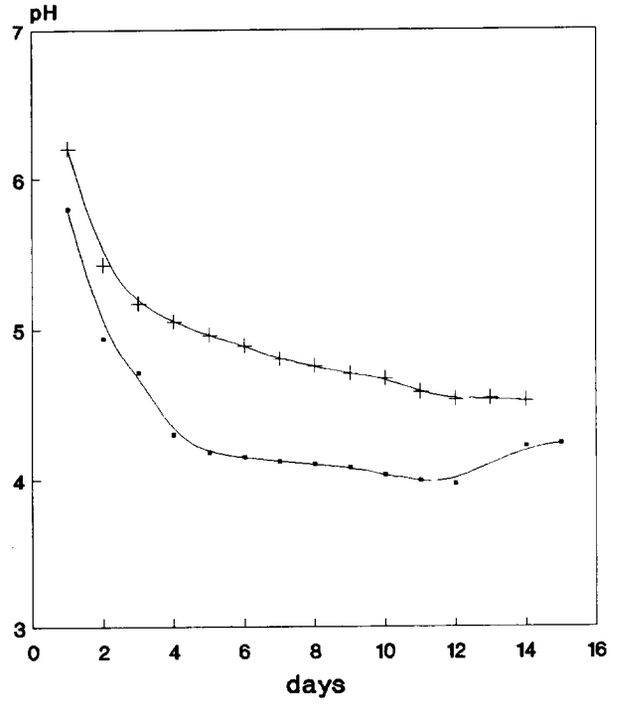


**Fig. 1.** pH pattern in the fermentation of fish waste by a combined starter culture of *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

## RESULTS AND DISCUSSION

### Chemical changes

The pH variations are plotted in Fig. 1. This showed a regular decrease from 5.8 to 4 after 5 days of fermentation for trial 1 and from 6.2 to 4.5 for trial 2. These values remained constant for 7 days and started to increase slightly to 4.2 for trial 1. The pH decrease in the product gives evidence of a good acidification through lactic acid fermentation by the starter culture of *Lactobacillus plantarum*.

The most important factor to control in the biotransformation is the pH decrease, which must be achieved as quickly as possible in order to inhibit the growth of spoilage microorganisms in the product. Moreover, the lactic acid fermentation is usually accompanied by some metabolites (bacteriocins), which may help in the preservation of the fermented foods.

The dry non-volatile matter (DNVM) variation patterns are plotted in Fig. 2. The decrease observed during the first stage of the fermentation may be due to the production of volatile compounds during the fermentation process. These compounds evaporate with water at 105°C during the determination of the dry matter and for this reason we called it 'dry non-volatile matter'. The breakdown of the organic matter by microoganisms and/or their enzymes is unavoidable during the process and may result in relatively high amounts of volatile compounds. In some cases and at the end of the process a slight increase—due to water evaporation—of the DNVM was observed, but this variation is not important.

An increase in the acid degree value (ADV) of the fat in the product was observed during the initial stage of

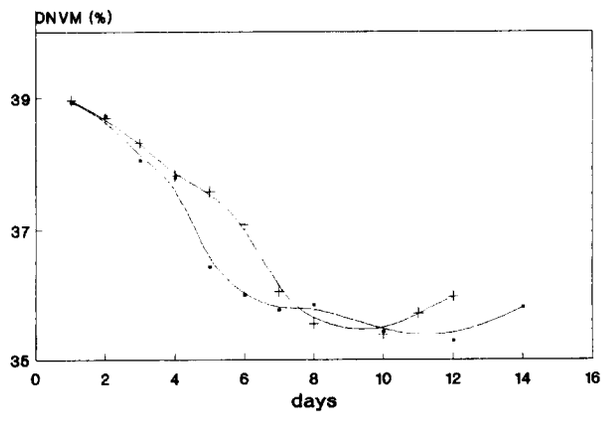


**Fig. 2.** Dry non-volatile matter change during the fermentation period of fish waste inoculated with *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

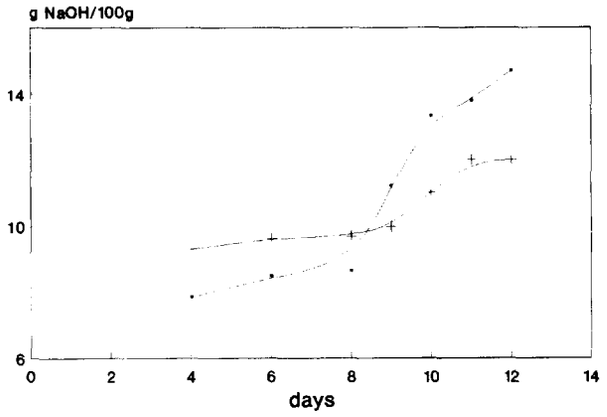


**Fig. 3.** Acid degree value of the fat from fish waste during fermentation by *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

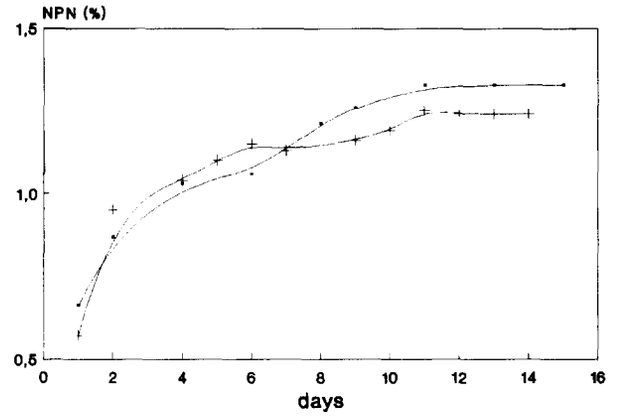


**Fig. 5.** Non-protein nitrogen change during the fermentation of fish waste inoculated with *Saccharomyces* and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

the fermentation (Fig. 3). The ADV increase may be due to lipid breakdown by lipolytic microorganisms and/or their lipases. This phenomenon is likely to occur during the first stage of the fermentation while the pH is still about neutral, so that lipolytic microorganisms can grow and consequently release their lipases.

The ADV in the fermenting product reached 13.2 mg $g^{-1}$ and 13.7 mg $g^{-1}$ for trials 1 and 2, respectively, and remained almost constant during the fermentation period. This may be explained by lipolysis being stopped by the inhibition of the lipolytic microbiota, or by the inhibition of the lipolysis reaction by the free fatty acids released in the medium during the fermentation, or by the environmental conditions produced by the fermentation (pH, aw, inhibitors). The TVN pattern in the product showed a slight increase during fermentation (Fig. 4). Haaland and Njaa (1990) found higher values of TVN in capelin stored 1 day before ensiling and in a silage stored for 7 days. These values were 3.1% and 2% of the TN, respectively. The rate of protein liquefaction is faster in acid silage than in fermented silage (Raa & Gildberg, 1982). In the present study, the TVN increased in trial 1 from 71.26 mg per 100 g (raw material) to reach 95.03 mg per 100 g after 1 day and remained constant around 132 mg per 100 g after 15 days of fermentation at 22°C. A similar profile was observed in the trial 2 for the TVN, which increased from 71.26 mg per 100 g to 141.4 mg per 100 g after 15 days. This was due to a fresh raw material which contained low levels of TVN and the addition of a starter culture which inhibited the growth of undesirable microorganisms, including proteolytics.

The NPN also increased notably in trial 1 during 11 days of fermentation (Fig. 5) to reach 1.3% and remained constant for the remaining period. A similar increase of NPN was observed in trial 2. The pattern of increase was controlled by the occurrence of lactic acid fermentation. A slight increase was observed throughout the entire period of fermentation. The NPN would indicate protein breakdown leading to release of amino acids and other metabolites originating from proteins.

The TMA pattern in the product during fermentation is plotted in Fig. 6. This shows a decrease of TMA in trial 2 from 8.08 mg per 100 g to 6.76 mg per 100 g during the first 5 days followed by a rapid increase between day 5 and day 9 to reach a maximum of 9.4 mg per 100 g. A

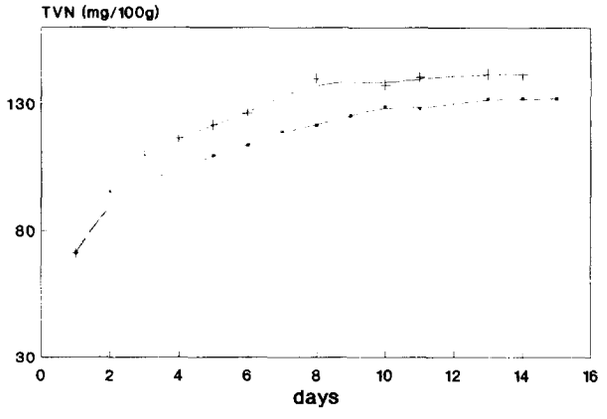


**Fig. 4.** Total vollatile nitrogen change during the fermentation of fish waste inoculated with *Saccharomyces* and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

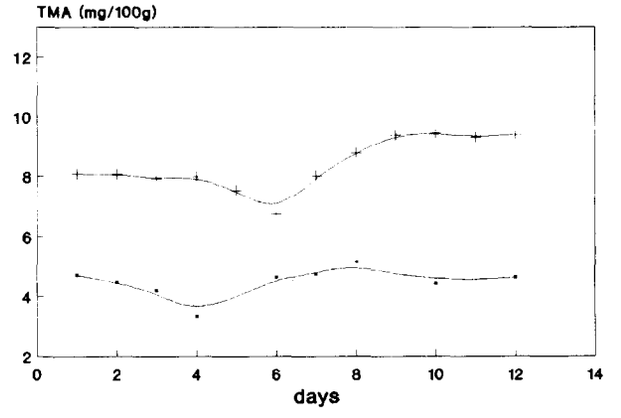


**Fig. 6.** Trimethylamine change during the fermentation of fish waste inoculated with *Saccharomyces* and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

similar pattern was observed for trial 1 but with lower values (from 4.7 to 4.64 mg per 100 g), thus indicating similar changes during fermentation.

No significant variation was observed during the last phase of the fermentation. This seems to be the result of the continuous removal by self-evaporation of the TMA produced; this is facilitated by production of gas by the yeast culture or to a delay in its formation by conditions being established in the product that are unfavourable for the microorganisms involved in transforming the protein into such compounds.

It would be interesting to learn more about the mechanism by which fish waste can not only be preserved but also by which the fish odor is removed. Knowledge of the chemical changes during the fermentation process that result in the disappearance of the fish-like odor could lead to the ingredient responsible being used at high proportions in animal feeds without any artefact. This could reduce the problem of scarce protein sources for animal feeds in Morocco by encouraging the conversion of fish waste into silage by using a low-cost process such as lactic acid fermentation.

A large increase in the TVN and the NPN was observed in the product, while the TMA was considerably reduced. This may be explained by the effect of the microorganisms during the fermentation process. Liquefaction may occur by the action of tissue-degrading enzymes (Raa & Gildberg, 1982), and the level of autolysis is related to activities of the digestive enzymes either present in the tissue or liberated by the contaminating proteolytic microorganisms.

## MICROBIOLOGICAL CHANGES

Microorganisms associated with food hygiene were monitored during the fermentation by the determination of coliforms and *Clostridium* counts. The microbial profiles are plotted in Fig. 7 for coliforms and Fig. 8 for *Clostridium*. Coliforms showed a net decrease during the fermentation to reach a minimum of $< 1\,\text{cfu}\,\text{g}^{-1}$ after 8 days for both trials. Counts remained constant for the remaining days of fermentation. The reduction of coliform numbers may ensure a good biopreservation against undesirable and/or hazardous microorganisms. *Clostridium* was not detected at high levels in the raw material ($< 10\,\text{cfu}\,\text{g}^{-1}$); the numbers did not increase in trial 1, but in trial 2 a slight increase was observed during the first day and then the count remained constant during fermentation. The low populations observed for *Clostridium* may indicate unfavourable conditions made by lactic acid fermentation.

Indicator microorganisms (coliforms) were eliminated in the product obtained after 3 days of fermentation. This could be due to the acidification and/or to some inhibitory compounds formed by the lactic acid bacteria. Owens and Mendoza (1985) reported that pathogens (*Salmonella*) and toxigenic microorganisms (*Clostridium* and *Staphylococcus*) are sensitive to a low pH. Moreover, fermentation by lactic acid bacteria can result in some inhibitors formed by these microorganisms and may ensure the safety of the product (this hypothesis has not yet been studied in fish silage).

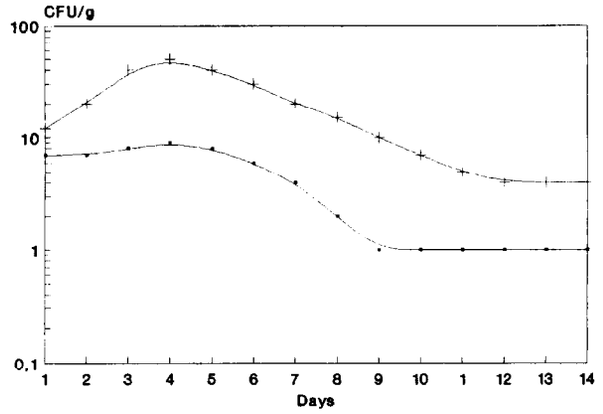


**Fig. 8.** *Clostridium* profiles in fish waste fermentation with pure cultures of *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

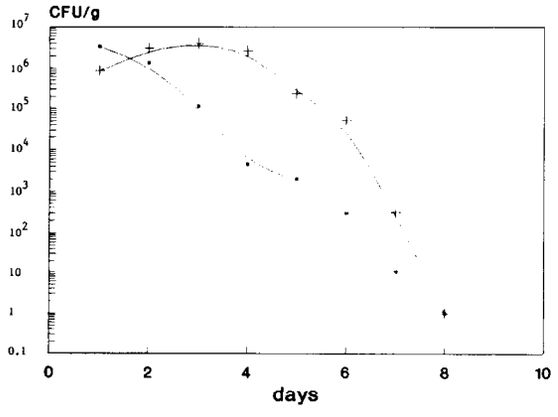


**Fig. 7.** Coliform profiles in fish waste fermentation with pure cultures of *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

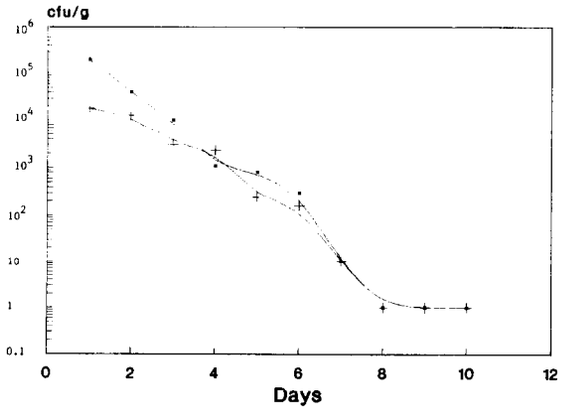


**Fig. 9.** Proteolytic microorganisms profiles in fish waste fermentation with pure cultures of *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

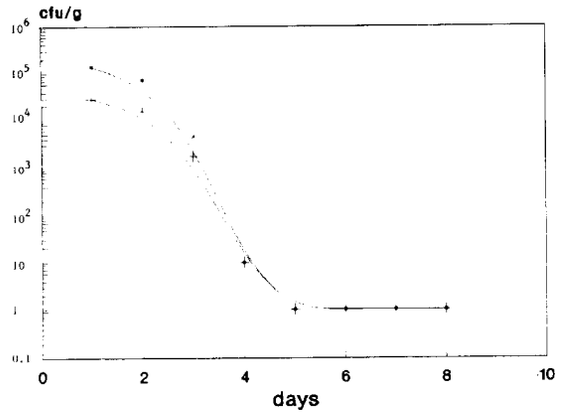


**Fig. 10.** Lipolytic microorganisms profiles in fish waste fermentation with pure cultures of *Saccharomyces* sp. and *Lactobacillus plantarum*. ■, trial 1; +, trial 2.

Results concerning the spoilage microorganisms pattern are reported in Figs 9 and 10 for proteolytics and lipolytics, respectively. Proteolytics show a first phase with a rapid slow-down to reach low levels after 5 days of fermentation. The same pattern was observed for the lipolytics, but the low level was reached after 8 days. The low level reached for both groups of microoganisms remained constant during the last period of fermentation (from day 8 to day 17) for both trials. The constant low level reached during fermentation may indicate a regular stability at this level and the success of the biopreservation of the fish waste against the undesirable biochemical breakdown of the organic matter leading to putrefaction of the product during storage.

Proteolytic and lipolytic microorganism inhibition in the product seems to be due to the low pH values and probably to the inhibitory metabolites formed by lactic acid bacteria in the medium during fermentation. It can be deduced that all the growth curves of the microorganisms studied presented an asymptotic part at the end of the process. This is because the method used started with a 1/10 dilution and no colony-forming units were detected in 10 ml of this dilution at the end of the process. Numbers were reported as $< 1$ cfu $g^{-1}$, but counts could be lower than what was reported.

Proteolysis may result in high amounts of TVN, NPN and TMA, and high levels of hydrolysis may lead to severe losses in the nutritional compounds in the product obtained. However, liquefaction due to the proteolytic enzymes may produce a good product which would contain available ingredients that could be used by the digestive system of the animal fed on the product.

## CONCLUSIONS

The occurrence of mixed fermentation by pure cultures of yeasts and lactic acid bacteria strains could be involved in both preservation, transformation and the improvement of the organoleptic quality of the obtained product. These parameters can be used to define a well-monitored process for the manufacture of an ingredient rich in nutrients which can be used in several feeds. Our results will be exploited in large-scale experiments by some factories to increase the value of their fish waste. Studies in this field are now being carried out and further data on the biotransformation of fish wastes and other solid or semi-solid waste into animal feed ingredients will be presented.

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