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Full Length Research Paper

Survey of the improvement of fish fermentation for *lanhouin* production in Benin

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The objective of this study was to control 'maturation' process of fish during the fermentation, by using bio-organisms that can give the expected improvement. Starter culture of *Lactobacillus plantarum* was used as bio-organism on species of fish such as *Pseudotolithus senegalensis* and *Scomberomorus tritor.* The inoculum was serially diluted (10^{-1} to 10^{-5}) in sterile Ringer solution. The result of this improved method showed significantly a product with a mild odour compared to odour from *lanhouin* traditionally obtained by chance fermentation.

Key words: Fermentation, fish, *Lactobacillus plantarum*, maturation, *lanhouin.*

INTRODUCTION

In Republic of Benin, fisheries produce fermented fish from some fish species. During the production, producers get losses of products because of unpleasant practices and uncontrolled phenomena. 'Food fermentation' is the study of microbial activity, usually anaerobic, on suitable substrates under controlled or uncontrolled conditions resulting in production of desirable foods or beverages that are characteristically more stable, palatable and nutritious than the raw substrate (Collins et al., 1993). Traditionally, many foods were prepared by fermentation but the reasons for the success or failure of the processes were not known. Now many food fermentation principles, and practices are well established, and food companies can predictably produce consistently good quality fermented product (Liu at al., 2009). With advances in genetic engineering, old processes are being improved and new ones are being discovered. Also many indigenous fermented foods, such as some oriental foods and African tribal foods, and their processes are not well known and are areas for future investigation (Barber et al., 1988).

Previous studies on bacteria, particularly Clostridia, showed activity in salted fermented fish (Metzler, 1977). Because of their high protein content fish constitute excellent media for putrefying bacteria. These organisms are found on the skin and in the viscera of the living fish. Putrefying bacteria multiply quickly and can spoil the food within a short time by proteolysis. But the use of the *Lactobacillus plantarum* for controlling the fermentation process is well appropriate (Annan et al., 2003; In and Mauguin, 1994). It has been known that the lactic acid bacteria do not affect the maturation process because they do not have proteolytic enzyme (protease) (Halm et al., 2004). But they are able to destroy putrefying microflora, inducing accumulation of the products of protein hydrolysis that gives the taste and aroma of the salted food commodity. The process that gives the fish a soft consistency, good texture and good flavour is usually named 'maturation'. With regard to biochemistry, maturation process is a protease process which renders the fish flesh tender and soft and generates the interaction between the products of amino acids) and the lipase thus, gives rise to the chains of amino acid and fat. These chains produce good taste and odour of the

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Table 1. Chemical characteristics of fish used for *lanhouin* production.

Fish	Content (%)			
	Protein	Lipid	Mineral substances	Moisture
Lesser African threadfin (*Galeoides decadactylus*)	20.4	1.4	12.0	77.0
Cassava croaker (*Pseudotolithus senegalensis*)	20.4	3.2	1.4	75.0
West African Spanish mackerel (*Scomberomorus tritor*)	23.1	10.1	1.5	65.3
West African ilisha (*Ilisha africana*)	20.0	22.0	2.0	56.0
Senegal jack (*Caranx senegallus*)	19.3	3.4	2.4	74.9

Sources: Russian encyclopedia of Technologist in fish industry, Part I, Moscow 1971.

mature product (Robinson, 1987). It has been established that the maturation process, its tendency and rate depend upon a number of factors as follows (Hui, 2005): (i) the nature of the substrate (fish species), (ii) the activities of the protease and lipase of the fish which change during the seasons of a year, (iii) the maturation temperature during the storage, characterized by the increase of the temperature which stimulates the maturation, the increase of the salt quantity slows down this process, and by (iv) the important role played by the substrate of the microflore in the protease action, concomitantly to fish. Acting on one or many of these factors one can stimulate the maturation of certain fish which alone are not able to mature (Dossou-Yovo, 2002). This paper aims at improving the techniques used traditionally with the view to correct some unpleasant practices and give innovative techniques which contribute to reduce nutrient and financial losses for the producers and to obtain a product with pleasant flavour, attractive texture and good shelf life.

MATERIALS AND METHODS

Inventory of traditional processing

Species used

Scomberomorus tritor (West African Spanish mackerel), *Ilisha africana* (West African ilisha) *Pseudotolithus senegalensis* (Cassava croaker), *Galeoides decadactylus* (Lesser African threadfin) *Caranx senegallus* (Senegal jack) and *Arius latiscutatus* (Rough-head sea catfish) were the species used in this study.

Species currently used

West African Spanish mackerel, lesser African threadfin and *Cassava croaker* these are species that are currently in used.

Indeed we use two species to observe the action of the lactic acid bacteria on the products without preliminary search on the predominant substrates'microflore. *L. plantarum* (starter culture) was collected from the University of Agriculture, Abeokuta. The idea was to improve fish fermentation process by using known organisms, and vis-à-vis two different species as lean and fatty species. Two samples of fish were used; the first sample was *Cassava croaker* (*P. senegalensis*) weighing (193.09 ± 0.01) g, and the second is West African Spanish mackerel (*S. tritor*) weighing (108.87 ± 0.01) g. The mackerel does not have scales, but the croaker has. The croaker was scaled and both fishes were washed with tap water. The inoculums *L. plantarum* was serially diluted (10^{-1} to 10^{-5}) in sterile Ringer solution (made by dissolving 1 tablet in 500 ml of distilled water), plated on sterile Plate Count Agar (PCA) – 20 ml, and was incubated at 37°C condition for 24 h for viability test. Then 1 ml of the viable inoculums (10^{-1}) dilution was used in inoculating the two species, respectively. The species were then incubated at ambient temperature (31°C) for 24 h to ferment. Sensory evaluation has been done in order to compare both samples to the traditionally obtained (Table 1) (Stone and Sidel, 1985).

RESULTS

Biochemical changes have been explored and have been noted in the Table 2 and 3. And microbiological changes of the samples subjected to an improvement have been recorded in the Table 4. Also, organoleptic indices have been appreciated (Table 5). Microbiological changes in traditional *lanhouin* have also been explored (Table 4). Same for organoleptical changes (Table 5).

DISCUSSION

After 20 h and 30 min (1230 min) the mackerel showed softening indices of fermented fish; the odour was not as objectionable as it is with from chance fermentation (Figure 1). For the croakers, the softening texture appeared after 21 h and 40 min (1300 min), but the tailwas more or less tough. After the fermentation period which is held at the ambient (31°C) temperature, the species was washed and salted in order to reduce microorganisms' activity and to reduce the maturation process of the salted fish. The maturation process has lasted for 48 h. The pH of the flesh of both fish species range from 6.41 to 6.05. The samples were dried for two (2) days, and the texture determined the duration of the sun-drying process. Figure 2 shows the different steps of the production of improved *lanhouin*. And it is important to mention that the drying process was held within an improved drying device that protects fish against infestations (Figures 2 to 6). The device is made of wood and inoxydable wire netting. For this modified processing

Table 2. Results of biochemical changes in improved *lanhouin.*

Parameter	Samples	
	Cassava croaker	Spanish mackerel
Moisture (%)	49.2 ± 0.27	41.4 ± 0.08
pH	6.41 ± 0.11	6.05 ± 0.07
Total volatile nitrogen (mg N/100 g)	161.7 ± 0.18	165.3 ± 0.09
Protein (%)	51.0 ± 0.25	54.2 ± 0.02
Lipide (%)	1.1 ± 0.01	7.6 ± 0.59
Salinity (g /100 g)	12.7 ± 1.20	11.8 ± 0.03

n = 3; (mean value ± standard error).

Table 3. Results of microbiological changes in improved *lanhouin.*

Parameter	Samples	
	Cassava croaker	Spanish mackerel
Aerobic bacteria	3.5×10^5 CFU/g	3.3×10^5 CFU/g
Yeats	None	None
Molds	None	None
Lactic flora (CFU/g)	$> 10^5$	$> 10^5$
Coliform bacteria	None	None
Escherichia coli	None	None
Fecal streptococci	143 CFU/g	141 CFU/g
Staphylococci	None	None
Micrococcus	1.0×10^3 CFU/g	1.1×10^3 CFU/g
Salmonellae	None	None
Anaerobic bacteria	none	None

Table 4. Microbiological changes in traditional *lanhouin.*

Parameter	Samples	
	Cassava croaker	Spanish mackerel
Total flora (CFU/g)	1.46×10^8	1.14×10^8
Lactic flora (CFU /g)	$< 10^6$	$< 10^6$
Total coliforms (CFU /g)	1200	$> 300 \times 10^2$
Fecal coliforms (CFU /g)	<10	<10
E. coli (CFU /g)	<10	<10
Anaerobic bacteria	<10	<10
Staphylococci	100	<100
Salmonellae	None/25 g	None/25 g

Table 5. Organoleptic indices of the samples after 22 h.

Parameter	Samples		
	Traditional sample	Cassava croaker	Spanish mackerel
Consistency	Rottenness	65% softness	80% softness
Colour	Blackish	No change	No change
Odour	Offensive	Not offensive	Not offensive

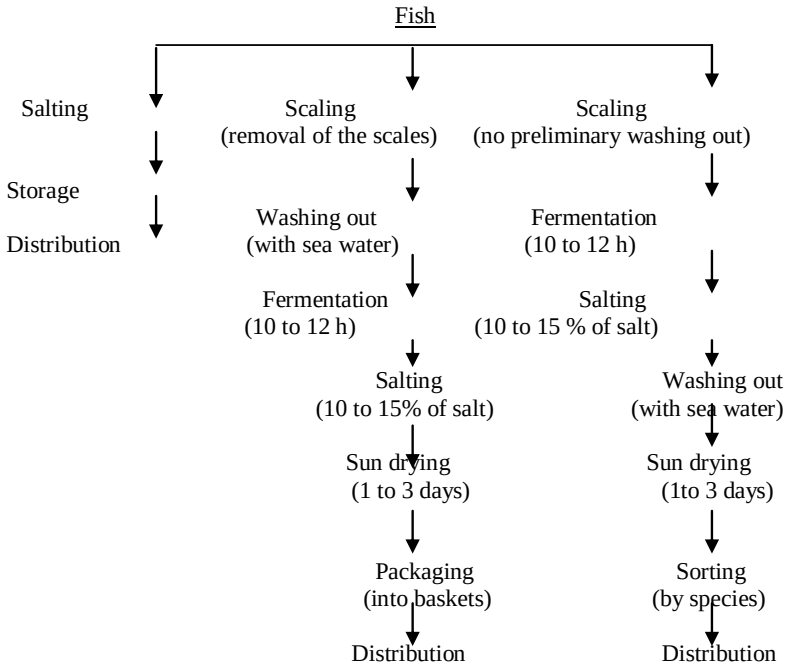

Figure 1. Flow chart of *Ianhouin* production in Benin.

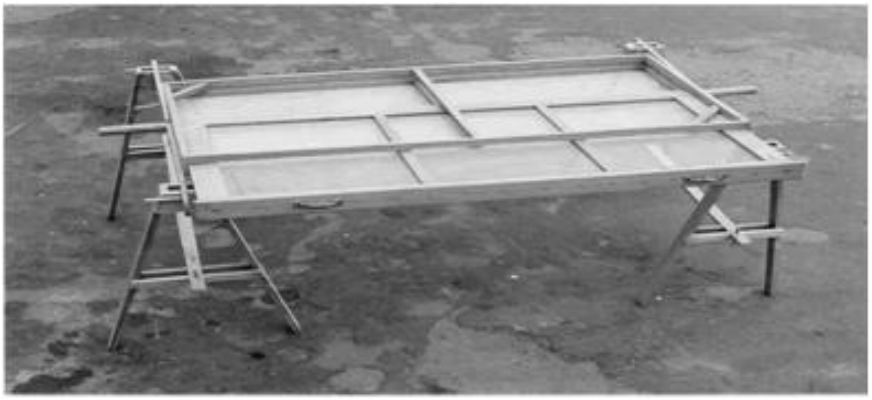

Figure 2. Opening of the device.

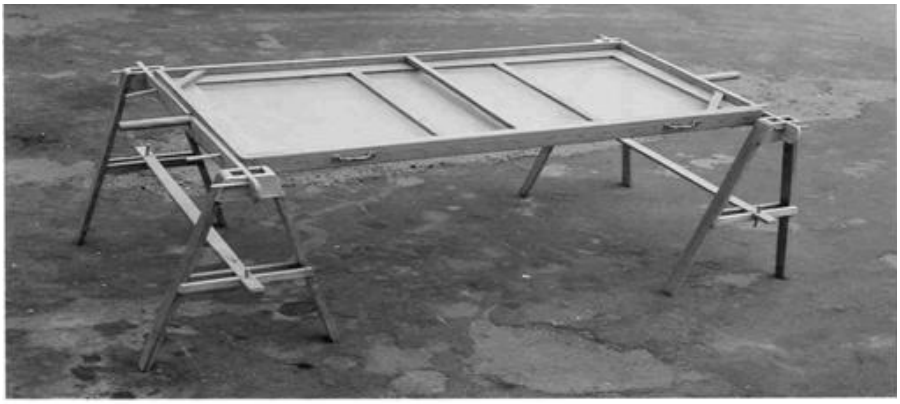

Figure 3. Shutting of the device.

Figure 4. Swiveling of the device.

Figure 5. Device swiveled.

Figure 6. Drying the fish with improved device.

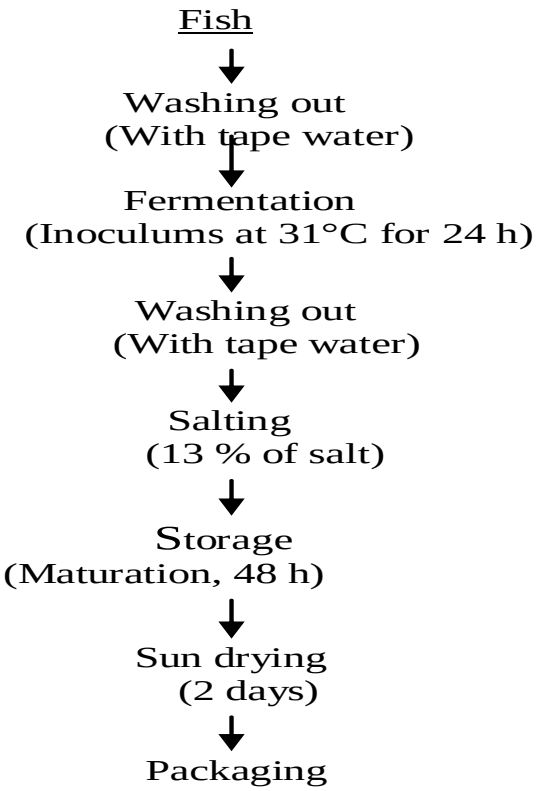

Figure 7. Flow chart of improved *lanhouin* production.

of *lanhouin*, it was observed that the product odour was mild as against the highly offensive odour from *lanhouin* obtained traditionally by chance fermentation (Table 5). But the rate of the fermentation process varies from one substrate to the other (1230 min against 1300 min), in spite of the use of the same concentration of inoculum product. This situation may be due to the difference in weight.

Conclusion

This study showed that fatty and less heavy fish, fermented more quickly than lean and heavier fish (first sample). Lactic acid bacteria were capable of inhibiting putrefying microflora, leading to a product with a good taste and aroma. Quantifying fermentation process, two (2) variables were shown to be used, the weight and the inoculums concentration, were according to the attribute (fish species) used.

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