

# Influence of Salt and Millet Treatments during Meat Fish Fermentation in Senegal

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## Abstract

Microbial growth in meat from traditional handled *Arius heudelotti* fish during fermentation at 25-30 °C in Senegal has been determined. Microorganisms involved in fermentation and pathogen microorganisms were analyzed in function of salt and millet addition [1/1 (w/v)].

Total viable microorganisms, lactic acid bacteria, H<sub>2</sub>S-producing Enterobacteriaceae, staphylococci, fungi and spore-forming bacteria counts in the crude meat fish reached  $6.34 \pm 0.28$ ,  $4.10 \pm 0.61$ ,  $4.33 \pm 0.45$ ,  $3.71 \pm 0.69$ ,  $1.50 \pm 0.3$  and  $1.33 \pm 0.58$  Log<sub>10</sub> CFU/g.

H<sub>2</sub>S-producing bacteria predominated ( $8.3 \pm 0.25$  Log<sub>10</sub> CFU/g) after 24 h incubation at 25-30 °C of untreated meat fish or that immersed in saline [14% NaCl (w/v)]. The pH of raw meat fish was  $6.32 \pm 0.1$ . It increased during unsalted fermentation, while it slightly decreased for the saline procedure. Meat fish fermentation in salty water added with NaCl at 80% (w/v), widespread in Senegal, allowed weak acidification in addition to growth inhibition of H<sub>2</sub>S producing Enterobacteriaceae which dropped to  $3.53 \pm 0.45$  Log<sub>10</sub> CFU/g after 24 h of fermentation. The fish fermentation in water added with malted millet flour at 15% (w/v) enabled significant growth of lactic acid bacteria and pH dropping to  $4.9 \pm 0.19$ . SH<sub>2</sub>-producing Enterobacteriaceae, Staphylococci and spore-forming bacteria showed a weak growth in the meat fish significantly acidified in millet solution, indicating preservative factor improvement when compared to the abusive salting traditional procedure which is a dietetic concern.

**Keywords:** fermentation, fish, millet, pathogens, salt, Senegal

## 1. Introduction

Marine source products significantly contribute to improve diet in Senegal, a Western African country where fisheries are of considerable economic, social and cultural importance. The annual fish production during the decade 2006-2015 reached 424,178 tons. The artisanal fishery, characterized by rare icing over fish handling, processing and marketing (Diei-Ouadi, 2005; Gram, 1992; Gram & Huss, 1996; GRET & CTA, 1993), drains 89.4% of the catches. Industrial fishery remains weakly developed and contributes for the rest (DPM,

2006-2015).

Because of different intrinsic specific parameters such as high water content, high level of protein or other nitrogenous constituents, and neutrophilic pH, fish is a very perishable resource. Both saltwater and freshwater fish are particularly sensitive to microbial contamination. Many fish spoilage bacteria are capable of good growth between 0 °C and 10 °C, justifying the importance of cold chain management to ensure safety and control organoleptic spoilage during crude fish handling or commercialization (Boyd, Green, & Lepors, 1992). The lack of adequate processing infrastructures or electricity in many Senegalese landing sites, contributes to significant post-harvest losses limiting the economic performances (Diei-Ouadi, 2005).

Fermentation, salting, drying and smoking or their combination, are the main technology used for the diversification of fisheries products in food preparation across the country (GRET & CTA, 1993). They significantly contribute to the reduction of post-harvest losses.

Guedj, a fermented and dried fish obtained by abusive salting, is one of the main indigenous processed fish products of Senegal. Guedj fish is comparable to lanhouin in Benin (Anihouvi, Hounhouigan & Ayenor, 2005), adjuevan in Côte d'Ivoire (Kouakou et al., 2013), Pla-soom in Thailand (Paludan-Müller et al., 2002.) and momoni in Ghana (Sanni, Asiedu & Ayernor, 2002).

Guedj fish is very popular in Senegal and in other West or Central African countries, due to its flavor (Fellows, 1997; GRET & CTA, 1993), justifying its widespread use as condiments in various culinary preparations across the country. Moreover, guedj contributes to furnishing more protein to the human diet in enclosed rural non-coastal areas rarely getting fresh fish supply.

Strategies for spoilage bacteria control during marine resources processing into indigenous seafood products are based on salt overuse. The process used to produce guedj fish remains the typical example of abusive salting, as preserving approach in Senegal.

Guedj fish processing involves three steps: fermentation, salting and sun drying. Two procedures are described for the step popularly called fermentation. One, older and presently marginal, is characterized by fish submission to fermentation at room temperature before being gutted, opened, abusively dry-salted and sun dried. The second approach nowadays more widespread is fish immersion in water with added salt (NaCl). The amount of NaCl added in the immersing preservative solution exceeds 30% (w/v) and can reach 80%. The immersed fish duration is 24 h to 48 h for flavor development. Subsequently, the fermented fish is dry-salted then dried under the sun for the end indigenous product (GRET & CTA, 1993; Infoconseil, 2005).

The efficiency of salt overuse in combination with 25-30 °C incubation to control undesirable microorganisms present in the fish during the fermentation as well as its potential negative effects on consumers are a dietetic concerns. Therefore, the search for an alternative fermentation reducing microbial spoilage during fish fermentation is a great challenge in Senegal artisanal fisheries. It will permit to meet food hygiene and safety requirements and reduce the need for abusive salt levels for guedj fish products.

Acidic pH values are mostly used to inhibit undesirable bacteria in food. It has been well established that most microorganisms grow best at pH value around 7.0 (6.6-7.5) whereas very few grow below 4.0 (Jay, Loessner & Golden, 2005). The most frequently reported genera of bacteria on raw and spoiled fish belong to Enterobacteriaceae or Pseudomonaceae families. The genera of *Bacillus*, *Escherichia*, *Enterococcus*, *Listeria* and *Clostridium* are known to occur on fish products. The minimum pH for the growth of *Aeromonas hydrophyla*, *Shewanella putrefaciens*, *Shigella flexneri*, *Clostridium botulinum G2* and *Pseudomonas fragi* is in the ranged of 5-6, while for *Escherichia coli O157:H7*, *Salmonella ssp.*, *Clostridium botulinum G1*, *Clostridium perfringens*, *Staphylococcus aureus* and *Listeria monocytogenes* the pH value is between 4 and 4.9 (Jay, Loessner & Golden, 2005).

When taking into account the technical and financial constraints in artisanal fisheries in Senegal, innovative technologies based on a fish significant acidification are the most suitable processing approach for improving preservative factors during fermentation at 25-30 °C. Among the microorganisms significantly present in artisanal handled fish, are lactic acid bacteria and Enterobacteriaceae, which both reach  $10^3$ - $10^4$  CFU/g (Diop et al., 2009). Psychrotrophic and mesophilic lactic acid bacteria present in the flesh of artisanal handled fish could contribute to its acidification during incubation at 25-30 °C by catabolism of fermentable sugars. These sugars must be externally furnished in the fish (Diop et al., 2009; Diop et al., 2015).

Millets constitute a cheap source of carbohydrates for the development of fish fermentation improvement strategies based on acidification using lactic acid bacteria. Malting contributes to increasing soluble carbohydrates content (Ndiaye et al., 2008; Taylor & Dewar, 2001).

This study aimed at measuring the capacity of meat fish acidification during incubation at ambient temperature, following immersion fermentation prevailing in Senegal for guedj, and assessing the impact by water added salt or millet to control microbial growth. The impact of salt and millet to improve meat fish preservation at ambient temperature (25-30 °C) contrasted with refrigeration technology used in modern Senegal fisheries to meet hygienic procedure (Gram & Huss, 1996). We determined the growth of spoiling fish microorganisms (Enterobacteriaceae, Staphylococci, spore-forming bacteria) and those contributing to fermented food taste or flavor such as Lactic acid Bacteria and Fungi.

## 2. Materials Studied

The study contributes to the development of technological approach limiting the growth of undesirable microorganisms during fermentation. The traditionally handled fish *Arius heudelotii*, a fatty fish (Diop et al., 2009) widespread amongst artisanal marine fishery landings in Senegal is one of the main species to be transformed into guedj.

## 3. Area Description

The analysis of traditional fermentation of fish for guedj making indicated a spontaneous fermentation with or without salt use (Gret & CTA, 1993). Moreover, refrigeration is nonexistent during the fermentation. The traditional spontaneous fermentation consists in allowing the decomposition of the raw fish matrix with or without salted water until a complete softening of the flesh. Incubation time and temperature for the spontaneous fermentation is varying between 24 and 48 h at 25-30 °C (ambient temperature), in prelude to dry salting followed by sun drying.

In these conditions, water activity and pH in the fish were the parameters on which preservative factors were based to control the growth of undesirable microorganisms in the traditional spontaneous fermentation justifying respectively salt and millet treatments applied in this study. Millet was the source of sugars. Effectively, during fermentation at ambient temperature, the pH of the fish flesh is a result of a complex equilibrium between the acidity produced by certain microbial groups including lactic acid bacteria capable of using sugars and the basic amino compounds produced from the hydrolysis of proteins.

The pH value ranging from neutral to basic may affect the quality of the targeted products (Yankah, 1988). They are particularly favorable for the development of some microorganisms such as Enterobacteriaceae during the fish exposure at ambient temperature similar to those prevailing in tropical areas. This group of bacteria may contain germs that cause bad odors or affect health (Jay, Loessner & Golden, 2005).

## 4. Methods and Techniques

### 4.1 Malting of Millet Seeds

Millet seeds (*Pennisetum glaucum*) were purchased at the local market of Ndar Tut in Saint-Louis. Fragmented seeds and foreign bodies were removed from the purchased sample. The whole millet grains were first disinfected in water by addition of sodium hypochlorite at 2% (w/v) for 10 min and rinsed with potable tap water. They were subsequently 3 times steeped in of tap potable water added with bicarbonate at 0.1% (w/v) at a ratio of 1: 2.5 (w/v) for 12 h (Ba et al., 2010). Then the water was drained and the washed grains kept for germination at 25-30 °C for 16 h. The sprouted seeds were spread on a blotting paper to remove excess moisture and dehydrated for 48 h by using a domestic electric tunnel dryer (Tompress, Sor èze, France).

### 4.2 Preparation of the Millet Flour and Solutions

The dehydrated lightly sprouted grains were ground to fine powder in an electric grinder. The water content of the flour was determined using a humidity balance (Sartorius MA 30).

The malted millet liquid matrixes were performed with flour added at 3, 7, 10, 15 and 20% (w/v) in 50 mL Falcon tubes. After vortex-homogenization (Heidolph, Reax 200, Bruckstr. 58 D-72393 Burlagdingen) for 5 min, the suspensions were decanted. One mL of the decanted solution was centrifuged (MicroStar MiniStar silverline, VWR). The supernatant was analyzed to determine the amount of total soluble substances using a refractometer (Optics Technology Delhi, India).

The solutions demonstrating a TSS varying between 0 and 1 °Brix were pasteurized at 80 °C for 10 min. They were cooled to reach 25-30 °C and subsequently tested as technological matrixes for fish fermentation by immersion procedure at the ratio 1.1 (w/v).

### 4.3 Source and Preparation of the Crude Meat Fish

Fish were purchased from the Sor local market in Saint-Louis (northern Senegal). They had a length of 30-40 cm

and their weight varied from 350 to 450 g.

Fish purchased in the evening from the different landing sites located in Saint-Louis, particular in of Nguet Ndar, are traditionally stored in baskets covered with ice overnight in the local market until the morning of the upcoming day for commercialization. Fish were scaled and eviscerated on site under the prevailing fish preparation conditions at the local marketing sites. Fish purchased by consumers are generally gutted on site by fish operators. This business activity enhances the risk of cross-contamination.

Gutted fish were brought to the laboratory within 15 min in plastic bags purchased on site and covered with ice during transportation. The eviscerated fish were rinsed in drinkable water in the laboratory. Filleting was done under sterile conditions. The yield was 2 filets reaching 110 g from each fish. The fillets were cut into small pieces up to 1.5 cm long, using a pair of scissors for the meat fish used in fermentation assays.

#### *4.4 Determination of the Millet Amount Required to Acidify Meat Fish*

The initial millet solution were prepared and analyzed as 4.2. The clear solutions demonstrating a TSS varying between 0 and 1 Brix were pasteurized by heating at 80 °C for 10 min. They were cooled to reach 25-30 °C and subsequently tested as technological matrixes for meat fish immersion at the ratio 1.1 (w/v). The treated fish were incubated at 25-30 °C for 24 h. The pH of the different millet treated meat fish with flour added was measured and compared to that of no treated meat fish for identification of those sowing pH dropping in the range [4.5 -5] considered as significant meat fish acidification.

#### *4.5 Determination of the Microbial Spoilage in the Raw Meat Fish*

Ten grams of unskinned flesh was removed from the crude fish pieces preparation under sterile conditions. They were suspended in 90 mL of sterile water containing peptone (0.1%) and NaCl (0.5%) in a sterile sealed plastic bag (BA 6141/CLR, Seward, Worthington, UK). The fish suspension was homogenized in a stomacher (Blender 80, Seward) for 2 min. The resulting homogenate was serially diluted in water containing peptone (0.1%) and NaCl (0.5%). One hundred (100) µL of each dilution was spread in triplicate on PCA supplemented with 0.5% NaCl for quantifying total viable microbial, MRS agar supplemented with 50 mg/L cycloheximide (Sigma, St. Louis, MO) and 100 UI/mL polymyxin b (Sigma) for LAB, Baird-Parker agar (Biokar Diagnostics, Beauvais, France) for staphylococci, and Hektoen enteric agar (Scharlau Chemie, Barcelona, Spain), for H<sub>2</sub>S producing Enterobacteriaceae.

Microscopic fungi (yeasts and molds) were counted by spreading 100 µL of the fish suspension on Sabouraud agar with chloramphenicol.

Colonies developing on the different plates were counted after 48 h of incubation at 30 °C (total viable microbial, microscopic fungi and LAB) or 37 °C (staphylococci, and H<sub>2</sub>S-producing Enterobacteriaceae).

#### *4.6 Determination of Spore-forming Bacteria in the Raw Meat Fish*

Spore-forming bacteria were quantified subsequently using a sporulation test performed by heat treatment. Two mL of fish suspension resulting from the stomacher (Blender 80, Seward) homogenization were heated at 80 °C for 10 min. The heat treatment was performed in a water bath (Nüve, bath 20 nb, Esenboga Yolu, Ankara Turkey). The heat treated fish suspension was cooled at 4 °C for 2 h. One hundred µL of the heated and subsequently cooled fish suspension were spread on PCA medium in triplicate. Colonies developing on the plates were counted after 40 h of incubation at 35 °C.

#### *4.7 Preparation of Salt and Millet Solutions Used for Immersive Fermentations*

Two NaCl concentrations were prepared and tested as immersion matrixes for salting fish fermentation assays at laboratory:

- high concentrated saline solution with 14% NaCl (w/v),
- oversaturated saline solution with 80% NaCl (w/v).

The masses of NaCl (Delta Salt, Infosa, Tarragona, Spain) required for achieving the target concentrations were added in drinkable tap water. Dissolution of salt in water was carried out using a magnetic stirrer (IKA C-MAG HS7 digital). The main preparations of each concentration were incubated at 80 °C for 10 min in a water bath (Nüve, bath 20 nb, Esenboga Yolu, Ankara Turkey) to kill potential vegetative microbial forms from the crude salt or water. The heated saline solutions were cooled to room temperature in prelude to immersed fish fermentation at the ratio 1:1 (w/v).

#### *4.8 Meat Fish Treatment for Fermentation at Ambient Temperature (25-30 °C)*

The total mass of raw meat fish were divided into 22 portions of 10 g each which were placed in the bottom of

sterile, disposable 50 mL Falcon tubes. The 22 tubes containing the pieces of raw fish were divided into 6 sets of two or four tubes. Table 1 gives the characteristics and repartition of the different sets.

Table 5. Repartition of the 22 Falcon tubes used for controlling the pH and microbial growth during the different fermentation procedures including treatment with salt or millet

Meat fish matrix and fermentation proceeding	Repartition of Falcon tubes containing meat fish			Total of tubes
	0h	24h	48h	
Crude meat fish	2	0	0	2
Untreated meat fish incubated at 25-30 °C	0	2	2	4
Meat fish immersed in water with NaCl added at 14% (w/v)] and incubated at 8 °C	0	2	2	4
Meat fish immersed in water with NaCl added at 14% (w/v)] and incubated at 25-30 °C	0	2	2	4
Meat fish immersed in water with NaCl added at 80% (w/v)] and incubated at 25-30 °C	0	2	2	4
Meat fish immersed in water with germinated millet added at 15% (w/v)] and incubated at 25-30 °C	0	2	2	4
Total	2	10	10	22

#### 4.9 Evolution of pH During Fish Fermentation Assays

In the case of non-salting fish fermentation, 10 mL of sterilized drinkable water was added to 10 g of fish in the 50 mL Falcon tube. The mixture was vortex-homogenized (Heidolph, Reax 200, Burlagdingen, Germany) for 5 min. The pH was measured by immersing the probe (Hanna Instruments 9125 pH meter) in the suspension at the different sampling times (0, 24 and 48 h).

When the fish was fermented by immersion in the water with NaCl added, the pH meter probe was directly immersed in the mixture which had been stirred vigorously by the vortex for 5 min (Heidolph, Reax 200, Burlagdingen, Germany), to determine the pH value at different times (24 or 48 h).

#### 4.10 Assessment of Microbial Growth in the Different Treated Meat Fish

When the fish was not salted during fermentation, 10 g of fish in a Falcon tube were suspended in 90 mL sterile water containing peptone (0.1%) and NaCl (0.5%) in a sterile sealed plastic bag (BA 6141/CLR, Seward, Worthington, UK) and homogenized in a stomacher (Blender 80, Seward) for 2 min for the first dilution. For salty fermentation, the first dilution was carried out by adding 10 g of fish in 10 mL of saline solution in 180 mL of sterile water containing peptone (0.1%) and NaCl (0.5%). All other procedures were the same.

For each fish fermentation procedure, the resulting homogenate was serially diluted in water containing peptone (0.1%) and NaCl (0.5%). The no-spore forming bacteria, fungi and spore forming bacteria in the different treated meat fish were assessed by direct plating using the previously described media after 24 and 48 h of incubation for fermentation.

#### 4.11 Repetition and Statistical Analysis

Each sample of raw millet and fermented meat fish was analyzed in triplicate and the figures were then averaged. Microbial counts and pH data of the different times of incubation (0 h, 24 h and 48 h) for the four fermentation procedures were assessed by analysis of variance (Anova), using The Stata ver.12.0 (Stata, Texas, USA) with a probability  $P \leq 0.05$ .

## 5. Results

### 5.1 Influence of Millet Germination on Total Soluble Substances

Pearl millet is the main cereal cultivated across the semi-arid lands in Senegal. It accounts for 66% of the total area of cereal cultivation across the country. The annual national millet production in Senegal was 698,643 tons in 2016-2017 (Direction de la Pr évision et des Etudes Economiques, 2017).

Grains of pearl millets contain 60-70% carbohydrates, 7-11% proteins, 1.5-5% fat, and 2-7% crude fibers (Kulther, Thorat & Lande, 2016). Germination is a cheap mean contributing to increase soluble carbohydrates content in pearl millet grain (Table 2) as demonstrated by Ndiaye et al. (2008).

Table 2. Details of specific carbohydrates composition of pearl millet

Components	Flour of ungerminated millet grain (g/100 g of grains)	Flour of germinated millet grain for 3 days (g/100 g of grains)	Source
Water content	8.72 ±0.31	8.74 ±0.18	Ndiaye et al., 2008
Starch	67.35 ±0.53	26.57 ±0.30	
Reducing sugar	1.33 ±0.22	8.72 ±0.80	
Total sugar	3.31 ±0.43	13.64 ±0.25	

Because of its availability in Senegal and the possibility of improving its technological potentiality by germination, pearl millet was the best candidate to improve fish fermentation at ambient temperature in Senegal by significant acidification (Diop et al., 2009; Ostergaard et al., 1998).

The total soluble substances (TSS) in the liquid phase obtained by using a hand refractometer was a preliminary indicator to evaluate the technological properties of millet used in fish fermentation. Such cheap approach could be reproduced without great constraint in small scale enterprises to control fish fermentation integrating immersion in millet as technological matrix.

Water content of the flour obtained from grounded dried millet was determined at 5.65 ± 0.37 %. The total soluble substances of the germinated and ungerminated millet solutions with flour added at 3, 7, 10, 15 and 20% (w/v) are presented in Table 3.

Table 3. Total soluble Substances (TSS) of solutions resulting from water added ungerminated and germinated\* millet

Parameters	Total soluble substances (TSS) in solutions from water added millet flour at 3 to 20% (w/v)				
	3	7	10	15	20
Ungerminated millet	0	0.20 ±0.00	0.67 ±0.12	0.93 ±0.12	1.13 ±0.12
Germinated millet	0	0.57 ±0.00	0.90 ±0.26	1.07 ±0.12	1.27 ±0.12

\*The germination of millet grains was performed at ambient temperature for 16 h

These results indicate that a millet solution with flour added over 3% enabled TSS quantification in using a hand refractometer. For each of the solutions with millet flour added at 7% to 20% (w/v), the TSS was higher when the millet grains had been preliminarily germinated (Table 3).

These results indicate that millet malting by germination at ambient temperature (25-30 °C) during at least 16 h contributed to enhance the nutrients in the grain which include fermentable sugars (Arora, Sudesh & Khetarpal, 2011; Ndiaye et al., 2008; Taylor & Dewar, 2001).

The yield of solution that will be used as immersion matrix for fish fermentation was considered as a second indicator of technological properties. The results of the influence of millet amount [0, 7, 10, 15, 20 and 25% (w/v)] added in the tap water, on the solution yield are shown in Table 4.

Table 4. Millet solution yield from water added with flour from germinated millet grains at range [0-25% (w/v)]

Parameters	Quantity of flour added to water % (w/v)					
	0	7	10	15	20	25
Yield of millet solution (mL)	100	73 ±5.65	62.5 ±0.71	49 ±0.00	33 ±1.41	18 ±2.83

These results combined to those of TSS predicted to use millet between 7 to 15% to get sufficient solution yield enabling fish immersion. When the millet flour was added in the tap water at amount over 15% (w/v), the solution yield was very low. In this case, the capacity of developing fish fermentation by immersion could be costlier.

### 5.2 Potential of Germinated Millet to Improve Meat Fish Acidification Capacity

The pH variation changes of untreated meat fish and samples immersed at the ratio 1/1 (w/v), in solutions obtained with tap water added malted millet flour at different concentrations, during incubation at 25-30 °C for 24 h are presented in the Table 4.

Table 5. Capacity of acidifying meat fish by immersion in preservative treatments. In solutions of water added with millet at different amounts [0, 7, 10, 15% (w/v)]

pH of crude and fermented meat fish	Quantity of flour added to water % (w/v) for fish preservative treatment			
	0	7	10	15
Initial pH of meat fish	6.41 ±0.21	6.41 ±0.21	6.41 ±0.21	6.41 ±0.21
pH of untreated and millet fermented meat fish incubated at 25-30 °C for 24h	6.51 ±0.16	5.75 ±0.14	5.26 ±0.05	4.96 ±0.08

These results indicate that a millet technological liquid matrix with flour added at 15% and ratio at 1/1 (w/v), was at least required to achieve significant pH dropping reaching value [4.5 - 5] of incubation at 25-30 °C for 24 h.

Such pH dropping level could contribute to decrease growth of certain neutrophilic microorganisms such as Enterobacteriaceae capable of spoiling meat fish incubated at temperature ranging over 10 °C (Diop et al., 2009; Gram & Huss, 1996).

Combination of refrigeration (4-8 °C) and salt addition permitted to meet good hygienic practice recommendations of highly salted seafood products and to control microbial growth during fermentation. However, this costly technology in energy justified its difficulty to be applied in Senegal. Moreover, the crude fish used for guedj in Senegal are generally advanced spoiled samples, such as those handled without refrigeration during commercialization in the local market for about 12 h at least. Therefore, we proposed a low-cost alternative fermentation technology to improve traditional fermentation of fish. This alternative technology, based on added malted millet flour at 15% (w/v), had the capacity to lower the main undesirable bacteria growing and spoiling meat fish during incubation at ambient temperature by a significant acidification enabled by fermentable sugar addition.

### 5.3 Influence of Millet and Salt on Meat Fish pH over Fermentation

The initial pH of the raw meat fish was  $6.29 \pm 0.03$ . The evolution of the meat fish pH during incubation without treatment (negative control) and immersion in saline water or malted millet are shown in Figure 1.

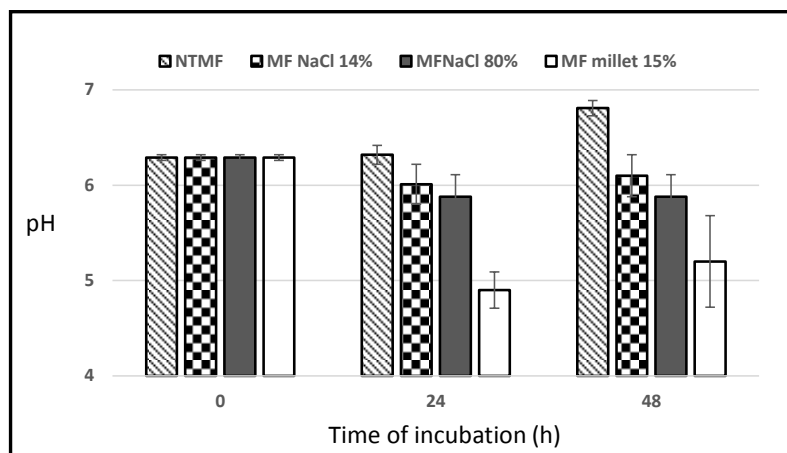


Figure 1. Influence of the meat fish preservative treatment in salt or millet on the pH\* during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MFNaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v)

\*: after 24 h of fermentation, a significant pH change ( $P < 0.05$ ) is occurred only for MF millet 15% (pH decrease).

The pH of the untreated meat fish increased slightly during incubation at 25-30 °C for 24 h, from  $6.29 \pm 0.03$  to

reach  $6.32 \pm 0.10$ . The saline immersion fermentations allowed a light pH dropping to reach  $6.01 \pm 0.21$  and  $5.88 \pm 0.23$  when the saline water was used with added salt respectively at 14 and 80% (w/v). The meat fish pH drops in millet flour solution added at 15% (w/v) were more significant than that of the two saline treatments after 24 h of incubation at 25-30 °C.

An increasing pH rebound was observed when the incubation of processed meat fish meat in the millet solution was extended to 48 h (Figure 1). This increase in pH can be explained by a predominance of amino acid metabolism in microorganisms present in fish after using all the fermentable carbohydrates provided by the millet treatment, which contributed to the early fall pH.

These results showed that the use of a millet solution at the ratio (1/1) having 1.07 °Brix as immersion matrix for meat fish fermentation, conducted to an acidification to  $\text{pH } 4.9 \pm 0.19$  during incubation at 25-30 °C for 24 h.

When the TSS of the millet solution increased to 1.5 °Brix by adding glucose at 0.5% (w/v) in prelude to the meat fish immersion, the pH dropped to 4.65 after incubation at 25-30 °C (data not shown).

#### 5.4 Influence of the Meat Fish Treatment with Millet or Salt on Microbial Growth

When considering the total viable microbial count of the four fermentation procedures (Figure 2), those without treatment of the meat fish and immersion in water added millet at 15% (w/v) allowed the highest growth.

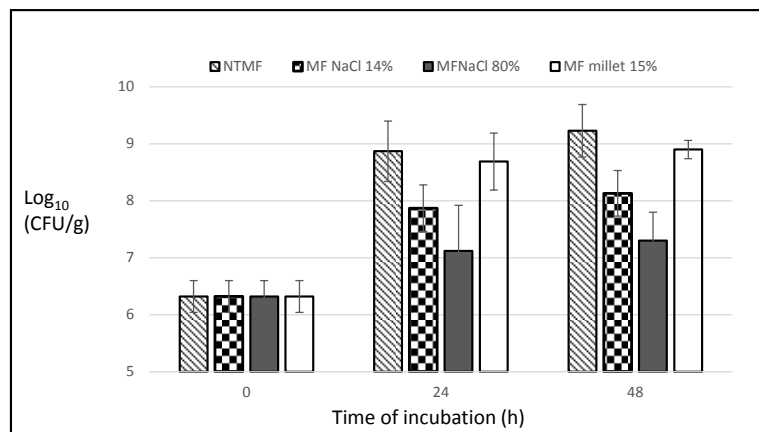


Figure 2. Influence of the meat fish preservative treatment in salt or millet on the total viable microbial growth during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MFNaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v)

\*: after 24h of fermentation, a significant growth of the total viable microbial ( $P < 0.05$ ) was occurred for NTMF, MF NaCl 14% and MF millet 15%.

The  $\text{SH}_2$ -producing Enterobacteriaceae grew significantly in the none-treated meat fish or that immersed in saline with NaCl added only at 14% (w/v) after incubation at 25-30 °C for 24 h to reach over 8 Log<sub>10</sub> CFU/g (Figure 3).



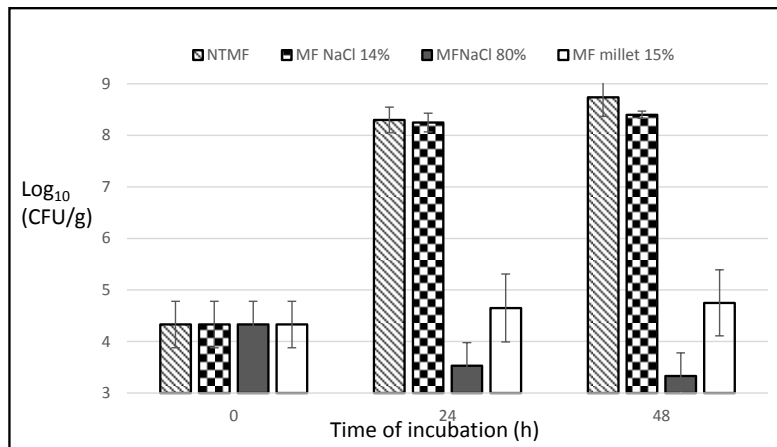


Figure 3. Influence of the meat fish preservative treatment in salt or millet on the SH<sub>2</sub>-producing Enterobacteriaceae growth during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MFNaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v)

\*: after 24 h of fermentation, a significant growth of the SH<sub>2</sub>-producing Enterobacteriaceae (P<0.05) was occurred for NTMF and MF NaCl 14%

The SH<sub>2</sub>-producing Enterobacteriaceae included pathogenic bacterial strains such as those belonging to the species *Salmonella sp* or *Escherichia coli*. When the fermentation was performed in water abusively added with salt at 80% (w/v) or millet, the capacity of these bacteria to grow has been significantly reduced. The number of SH<sub>2</sub>-Producing Enterobacteriaceae in the meat fish treated following the two last procedures was lower at 3.65 to 4.77 Log<sub>10</sub> CFU/g than those determined on the non-treated or immersed in water added NaCl at 14% (w/v).

The abusive salt preservative treatment of the meat fish is a dietetic concern justifying the importance of fermentation in millet as alternative approach. The last fermentation procedure allows significant acidification that affects growth potential of SH<sub>2</sub>-producing bacteria.

The staphylococci were the second bacterial population demonstrating a significant differential growth potential following the fermentation procedures. They significantly grew during meat fish fermentation at 25-30 °C without treatment or immersion in saline with NaCl added only at 14%, compared to those in abusive salt preservative treatment or millet (Figure 4).

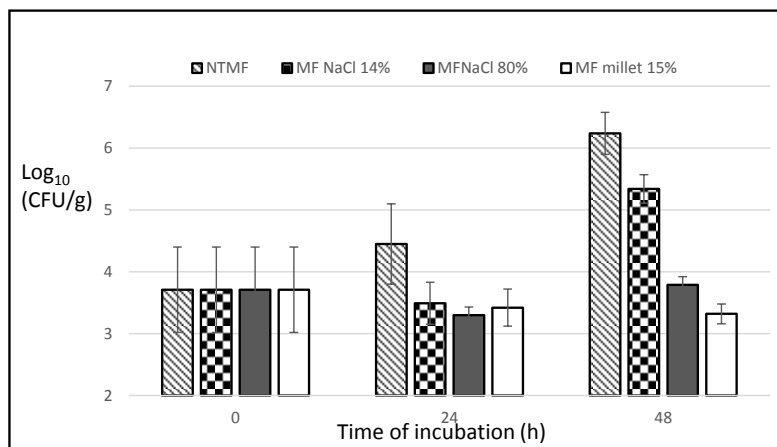


Figure 4. Influence of the meat fish preservative treatment in salt or millet on the Staphylococci growth during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MFNaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v).

\*: after 48h of fermentation, a significant growth of the staphylococci ( $P < 0.05$ ) was occurred for NTMF and MF NaCl 14%.

Compared to  $SH_2$ -producing Enterobacteriaceae, the significant growth of this bacterial population occurred lately, after 48 h of fermentation at 25-30 °C (Figure 4).

The spore-forming bacteria showed a significant lower growth potential in the meat fish during the 4 fermentation procedures amongst undesirable bacteria targeted in the study including  $SH_2$ -producing Enterobacteriaceae and staphylococci (Data not shown).

Lactic acid bacteria (Figure 5) and fungi (Figure 6) which can both contribute to the flavor of fermented food products, showed differential growth potential following the preservative treatments.

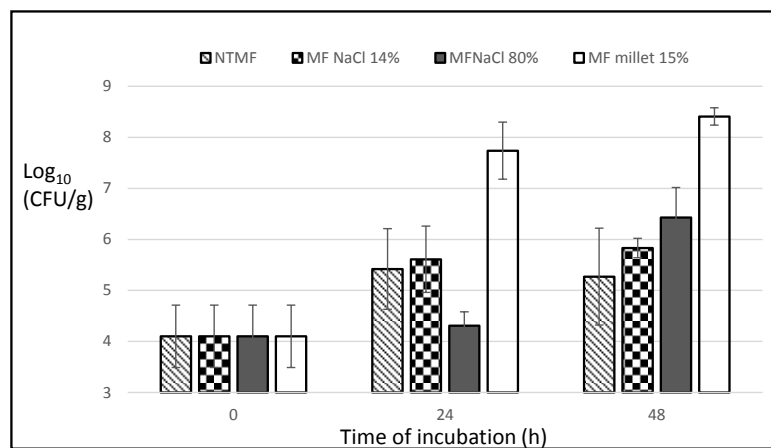


Figure 5. Influence of the meat fish preservative treatment in salt or millet on the Lactic acid bacteria growth during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MF NaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v)

\*: after 24h of fermentation, a significant growth of the lactic acid bacteria ( $P < 0.05$ ) was occurred for MF millet 15%.

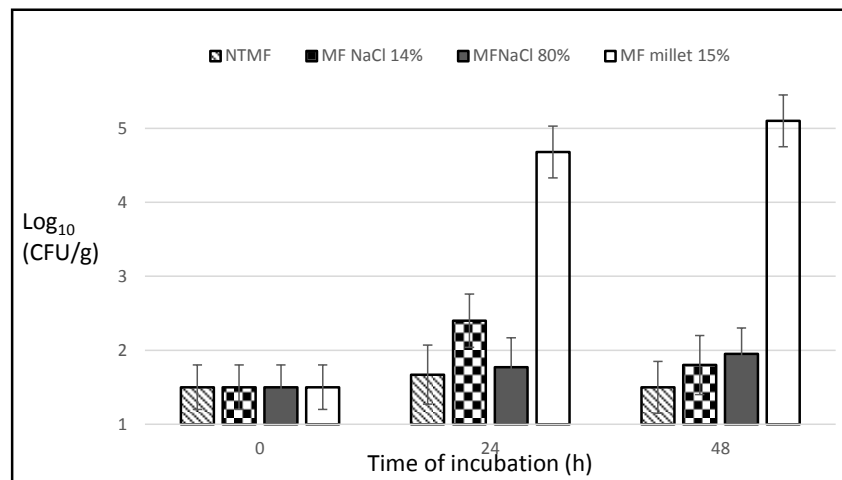


Figure 6. Influence of the meat fish preservative treatment in salt or millet on the fungi growth during fermentation at 25-30 °C

NTMF: not treated meat fish;

MF NaCl 14%: meat fish fermented in saline with NaCl added at 14% (w/v) by immersion at a ratio 1/1 (w/v)

MF NaCl 80%: meat fish fermented in saline with NaCl added at 80% (w/v) by immersion at a ratio 1/1 (w/v)

MF Millet 15%: meat fish fermented in malted millet solution with flour added at 15% (w/v) by immersion at a ratio 1/1 (w/v)

\*: after 24h of fermentation, a significant growth of the fungi ( $P < 0.05$ ) was occurred for MF NaCl 14% and MF millet 15%.

The number of fungi and lactic acid bacteria increased from  $1.5 \pm 0.3$  and  $4.1 \pm 0.61$  Log<sub>10</sub> (CFU/g) respectively to reach  $4.68 \pm 0.35$  and  $7.74 \pm 0.56$  Log<sub>10</sub> (CFU/g) in the significantly acidified meat fish characterizing millet preservative treatment, after incubation at 25-30 °C during 24 h. The growth of these two microbial populations was significantly lesser in the untreated or salted meat fish during their fermentation.

## 6. Discussions

Indigenous seafood products contribute to cover the demand of animal protein source foods in Senegal and many other sub-Saharan countries (Fall et al., 2017; Fall et al., 2018; GRET & CTA, 1993, Infoconseil, 2005; Anihouvi, Hounhouigan & Ayenor, 2005; Anihouvi et al., 2006; Fellows, 1997). They constitute a major food resource in the non-coastal areas. Strengthening the contribution of these resources to food security and economic growth requires an evolution of traditional fisheries systems.

This development involves changes in post-harvest treatment practices, improved technologies that significantly reduce salt misuse, and information on the products quality relative to the nutritional and hedonic requirements of consumers. The quality of the products must be based on a better knowledge of biochemistry, microbiology and the labeling of fish products.

Regarding the fermentation of fish in Senegal, the application of refrigeration (4-8 °C) combined with regulatory salting (*Codex alimentarius*, 2012) as technological means, meeting hygienic recommendation to control undesirable bacteria growth during processing, is non-existent. The cost of the investments required for the refrigeration and that of the electric energy consumption are two examples of constraints limiting the use of modern preservative technologies.

The major technological challenge of innovation to improve preservation factors during fish fermentation in Senegal is based on the substitution of the traditional salty solutions used during the incubation at 25-30 °C for flavor development in prelude to sun drying. This study has shown that SH<sub>2</sub>-producing Enterobacteriaceae and Staphylococci (Figures 3 and 4) are amongst the main undesirable microorganisms that could be controlled to contribute to the hygiene of fish fermentation at 25-30 °C which is prevailing in Senegal (Fall et al., 2017; Fall et al., 2018). An abusive salting by using water added with 80% NaCl allows their growth control, but it remains a dietetic concern.

Control of these bacteria growth can be achieved by significant acidification of the meat fish. Matrix supplying sufficient fermentable carbohydrate corresponding to a TSS range of at least 1 °Brix such as water added malted millet flour at 15% (w/v) could be used. Such matrix can be a credible alternative to reduce salt in fermented seafood processed in Senegal.

Millet is a locally available grain and its malting is achievable at room temperature for enhancing the total sugar content. It could be used to enhance the external supply of fermentable sugar in the immersed fish. The cost of this technology is cheap enough to correspond to the financial capacities of the small-scale enterprises producing fermented fish across the country.

In addition, the specific microbial content in the millet immersed fermented fish meat showed a quantitative predominance of lactic acid bacteria (Figure 5). LAB originally isolated from foods are the best candidates for improving the microbiological safety of these foods because they are well adapted to food conditions and should therefore be more competitive than LAB isolated from other sources. The strain population of Lactic acid bacteria massively present on millet acidified meat fish could include bacterial strains developing other complementary antimicrobial properties (Savadogo et al., 2004; Meera & Devi, 2012). They could be used as starter culture for improving the fish fermentation at 25-30 °C (Anihouvi et al., 2012; Diop et al., 2009; Diop et al., 2015; Fall et al., 2018; Bagenda et al., 2008).

The results of this study justify to continue to characterize the specific lactic acid bacteria massively present in significantly acidified fish meat on millet solution in order to identify those showing different potentiality of antimicrobial actions, which will be the best candidates for a starter culture development.

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