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Improvement of Taste Enhancer Condiment Processing and Safety Using Marinade and bio-preservation of Cassava Fish (*Pseudotolithus* Sp)

Janvier Melegnonfan Kindossi[a,b], Ogouyôm Herbert Iko Afé[a], Générose Vieira-Dalodé[b], Noël Houédougbé Akissoé[b], Sabine Leroy[c], Régine Talon[c], Victor Bienvenu Anihouvi[b], and Djidjoho Joseph Hounhouigan[b]

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ABSTRACT

This study aims to improve Lanhouin production using marinade and mixed starter cultures of *Lactobacillus plantarum* and *Staphylococcus xylosus* as bio-preservation agents. The microbial and the physico-chemical properties of fish products obtained were characterized according to standard methods. Both combined strains developed an excellent ability to ferment fish flesh. The samples inoculated with the starter cultures had low pH value (4.7) and high level of lactic acid bacteria (LAB) (6.2 Log CFU/g) and coagulase negative *Staphylococcus* (CNS) (5.4 Log CFU/g) than the control samples. After 90 days of storage, the ratio of total viable count (TVC) and LAB count in the final dried products was equal to 100, as recommended by the "Federation of Trade and Distribution Companies" (FCD). Moreover, no pathogens were detected in the analyzed samples, and yeast and molds counts were less than 1 Log CFU/g.

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Fish; marinating; fermentation; *Lactobacillus plantarum*; *Staphylococcus xylosus*; flavoring agent

Introduction

Fish and fishery products contribute to ensuring food security by their micronutrients, essential fatty acids and proteins and also by guaranteeing a source of income to the fishing communities and the state (FAO, 2007; Iko Afé et al., 2020). It is estimated that fish contributes up to 180 kilocalories per person per day, but reaches such high level only in few countries where there is a lack of alternative foods (FAO, 2007). However, preservation of fish in the tropical countries is difficult due to its extreme perishability, the lack of adequate infrastructures of preservation, the climatic conditions and the poor handling which favor its spoilage in few hours (Kindossi et al., 2012). Fish post-capture loss can be limited by developing processing methods such as drying, smoking and fermentation sometimes in combination with salting (Anihouvi, Kindossi,

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& Hounhouigan, 2012; Ezeama, 2012; Kindossi et al., 2012). Natural fermentation is one of the processing methods mostly used over the world in combination with salting followed sometimes by drying (Wang, Xia, Gao, Xu, & Jiang, 2017). In Benin, Lanhouin, a traditional flavoring agent and taste enhancer, is an example of fish product obtained by natural fermentation (Anihouvi, kindossi & hounhouigan, 2012; Kindossi et al., 2013). The disadvantages of this type of fermentation are related to the fact that no control can be exerted on the process. Consequently, the final product is often of variable quality with risks of quality defect and formation of toxic compounds such as histamine (Kindossi et al., 2016a). To avoid safety issues associated with traditional Lanhouin (Kindossi et al., 2012), food preservatives can be used to create hostile environment to the growth of spoilage and pathogenic microorganisms by shortening their survival or causing their death (Dalgaard, 2000; Fernandes, 2009; Leistner, 2000). Among food preservation technologies, particular attention has been given to the bio-preservation to extend the shelf-life and to enhance the hygienic quality, minimizing the impact on the nutritional and organoleptic properties of perishable food products such as seafoods (Cortesi, Panebianco, Giuffrida, & Anastasio, 2009; Dalgaard, 2000; Fernandes, 2009; Leistner, 2000; Leroi et al., 2006; Soomro, Masud, & Anwaar, 2002). The bio-preservation is a process which consists of inoculating a food product with strains of bacteria selected so as to inhibit the growth of the undesirable flora without modifying the organoleptic characteristics of this product (Fernandes, 2009; Khusro, Aarti, Salem, & Barbabosa-Pilego, 2020; Lacroix, 2011; Rodgers, 2001). However, contrary to natural fermentation which is one of the oldest bio-preservation technologies is followed by a significant transformation of the organoleptic characteristics of the final product due to the presence of high potential undesirable flora which affected its quality (Fernandes, 2009; Lacroix, 2011; Leroi et al., 2006). Various mechanisms are involved in bio-preservation technology. The inhibition of the undesirable flora by bioprotective bacteria can be due to the nutritional competition during growth and to the presence of certain products of their metabolism or to the production of bacteriocins (Bao et al., 2018; Fernandes, 2009; Galanakis, 2019; Kumar et al., 2017; Lacroix, 2011; Leroi et al., 2006). Bacteriocins are antibacterial proteins secreted by lactic acid bacteria. They display variable antibacterial activity against food-borne pathogens (Bao et al., 2018; Kumar et al., 2017; Lacroix, 2011). The bio-preservation studies were especially focused on the bacteria that are able to degrade histamine by the production of amine oxidase. Thus, a strain of *Staphylococcus xylosus* inoculated in preparations of salted and fermented anchovies is able to degrade 38% of the histamine produced in fermented anchovies (Mah & Hwang, 2009a). Also, the marinating was proved to reduce significantly the aerobic flora (Kindossi, Anihouvi, Vieira-Dalodé, Akissoé, & Hounhouigan, 2016) and to extend considerably the shelf life of vacuum and

cooled (4°C) packed fish (Sallam, 2008). Moreover, Kin et al. (2011) reported that the marinating of the fish fillet with salt, phosphate and a combination of acetate of potassium and lactate inhibited the development of the psychrotrophic bacteria of fish fillets cooled at 4°C and improved the sensory characteristics of the final products. But, the combined effect of bio-preservation and marinating was not tested yet during Lanhouin production. In addition, the previous studies on the controlled fermentation of Lanhouin (Anihouvi, Kpoclou, & Hounhouigan, 2012) did not integrate the combined use of microorganism strains which are able to reduce the content of biogenic amines and those which are able to improve the flavor. This study aims to develop an improved processing of fish into Lanhouin using marinade during ripening step associated with bio-preservation of fish flesh with strains of *Lactobacillus plantarum* and *Staphylococcus xylosus.*

Materials and methods

Fish and marinade solution

Fresh cassava fish (*Pseudotolithus* sp.) was purchased from Cotonou (Benin) seaport. The fish was transported to the Laboratory in an icebox with dry ice. This type of fish was chosen due to its general use to produce artisanal Lanhouin (Kindossi et al., 2012).

Marinade solutions were prepared with 1% of extra pure citric acid (AC07200500, Scharlau Chemie S.A., EC Label, Spain European Union), 10% of Laboratory sodium chloride (NaCl) (GPR rectapur 11G130020 EC Label, European Union), and 0.5% of spices (garlic, clove, black pepper) (purchased from local supermarkets) and distilled water.

Strains of microorganisms

Lactobacillus plantarum (LP 652) and *Staphylococcus xylosus* (SO3-188) were provided by the laboratories of CIRAD-Montpellier and INRA-Clermont-Ferrand Theix (France), respectively.

Preparation of fish

The fresh cassava fish was washed, scaled, gutted, beheaded and washed again before filleting. The fish flesh was separated from skin and bones and then marinated for 4 h. The marinated flesh was ground using a mixer blender (type 1985/R20, Wodschow, Denmark). The marinated ground flesh was mixed with 5% of NaCl and 5% of sterilized cassava (*Manihot esculenta crantz*) starch powder as fermentable carbohydrate source for *Staphylococcus xylosus* and *Lactobacillus plantarum* (Figure 1).

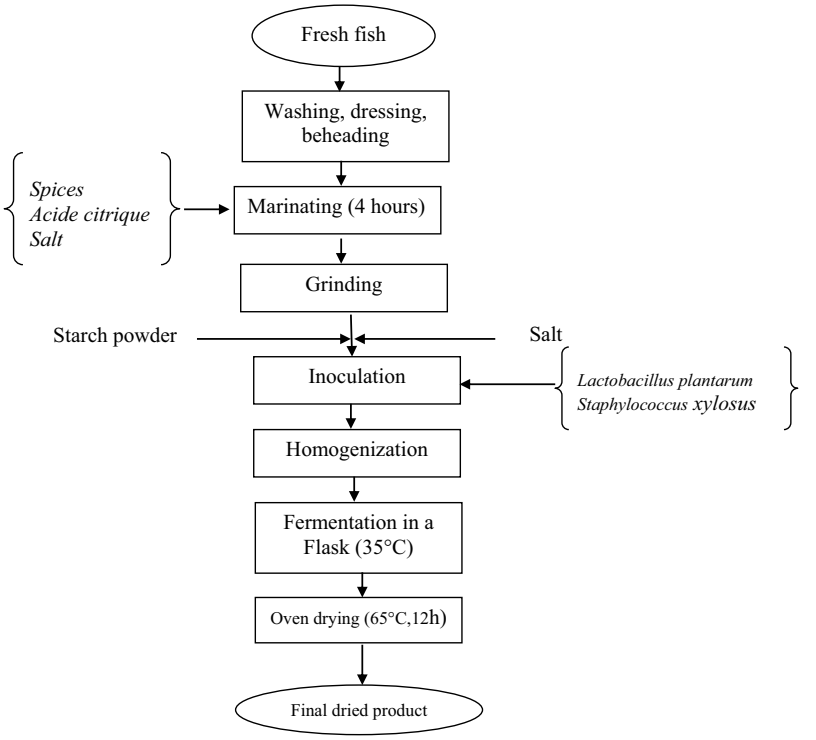

Figure 1. Process flow diagram for the production of final dried product.

Preparation of inocula

Staphylococcus xylosus (SO3-188) and *Lactobacillus plantarum* (LP 652) were cultivated by streaking onto Nutrient Agar (NA, Oxoid CM 0005, Basingstone, Hampshire, England) and MRS Agar (MRS, Oxoid CM 0361, Basingstoke, Hampshire, England), and incubated at 37°C and 30°C for 24 h and 72 h, respectively. One colony of each micro-organism was then picked and inoculated to 10 ml of Nutrient broth (NB, Oxoid CM 0001, Basingstone, Hampshire, England) and 10 ml of MRS broth and incubated during 24 h at 30°C and 37°C for *S. xylosus* and *L. plantarum*, respectively. From each culture, 0.1 ml was inoculated in 10 ml Nutrient Broth or 10 ml MRS broth according to each strain incubated at 37°C and 30°C for 16 h, respectively, and then centrifuged at 3000 × g for 10 min.

The pellet collected was washed twice in 10 ml of sterile physiological water [peptone 1 g (Oxoid LP 0037, Basingstoke, Hampshire, England), NaCl 8.5 g in 1000 ml of distilled water with pH = 7.2 and centrifuged again. The pellet was then diluted in sterile peptone water to obtain approximately a concentration of 10^6 cells/ml of suspension, tested by culture on Nutrient Agar (NA, Oxoid CM 0005, Basingstone, Hampshire, England) for *S. xylosus* and MRS Agar (MRS, Oxoïd CM 0361, Basingstoke, Hampshire, England) for *L plantarum*.

Inoculation of marinated fish flesh mix

The marinated fish flesh mix was inoculated with a mixture of starter cultures of *L. plantarum* and *Staphylococcus xylosus* and homogenized in aseptic conditions. For the mixed starter cultures, the suspensions of both cultures were mixed equally and 1 ml of the final mixed culture was inoculated into 100 g of marinated fish flesh (this gave approximately a concentration of 106 cells/g of fish flesh). The mixed flesh fish was aseptically packaged into 250-ml sterilized flask. To avoid disturbing the fermentation during sampling, one flask was prepared for each sampling time. Each inoculated sample was aseptically mixed with sterilized spatula, then covered and incubated at 35°C for 36 h. Non-inoculated samples were also made and used as a control for each sampling time.

Sampling

The sampling was composed of two independent batches (control and inoculated samples), with five replicates per batch at each sampling time. Sampling was done at 0, 6, 12, 24 and 36 h of fermentation for microbiological, physicochemical analysis. In total, five samples, each representing one fermentation time, were fermented at two different occasions using the combined starter culture of *L. plantarum* and *Staphylococcus xylosus* and non-inoculated samples (control).

Evaluation of the shelf life of biopreserved fish flesh after drying

The final products were oven-dried at 65°C during 12 hours and then were ground using a blender (Waring Commercial Blender 35B64, USA). Finally, the products were presented in powder form. The biopreserved fish flesh samples and the non-inoculated samples were dried at 65°C during 12 h to obtain a moisture content of about 7.5%. The dried samples were packaged in plastic bags (Type Walovac 90 B) and stored at 30 ± 2°C for 90 days.

Microbiological analysis

Ten (10) g of each sample were introduced aseptically in a sterile stomacher bag and 90 ml of sterile diluent containing 0.1% peptone (Oxoid L37, Basingstoke, Hampshire, England), 0.8% sodium chloride (NaCl) (Merck KGaA, Germany) with pH adjusted to 7.2 was added. The mixture was then homogenized for 2 min using a Stomacher (Lab-Blender, Model 80, Seward Medical, and London, UK) (ISO-6887-1, ISO-6887–1, 1999). One ml of the suspension was serially diluted and used for microbial counts according to ISO norms. Total viable counts (TVC) were enumerated using Plate Count Agar (PCA, Oxoid CM0325,

Basingstoke, Hampshire, England), and PCA plates were incubated at 30°C for 72 h (ISO-4833, ISO-4833, 2003) . Lactic Acid Bacteria (LAB) were enumerated using de Man, Rogosa, Sharpe agar (MRS, Oxoid CM0361, Basingstoke, Hampshire, England). The MRS plates were incubated at 30°C for 72 h (ISO-15214, ISO-15214, 1998). *Enterobacteriaceae* were enumerated using Violet Red Bile Glucose Agar (VRBG, Oxoid, CM0485, Basingstoke, Hampshire, England), and the plates were incubated at 37°C for 24 h (ISO-21528, ISO-21528, 2004). Yeasts and molds were enumerated using Yeast Extract Agar (Oxoid CM0019, Basingstoke, Hampshire, England) supplemented with chloramphenicol (Oxoid SR0078E, Basingstoke, Hampshire, England) and the inoculated plates were incubated at 25°C for 3–5 days (ISO-7954, ISO-7954, 1988). *Staphylococcus aureus* and coagulase negative *Staphylococcus* were enumerated using Baird Parker agar base (Oxoid CM0275, Basingstoke, Hampshire, England) supplemented with egg yolk tellurite emulsion (SR54, Basingstoke, Hampshire, England). The inoculated plates were incubated at 37°C for 24 h (ISO-6888, ISO-6888, 1999).

Physico-chemical analysis

pH, water activity (a_w), moisture, total volatile nitrogen (TVN), and biogenic amines (histamine, cadaverine, putrescine and spermidine) of samples were determined according to the method described in the previous work (Kindossi et al., 2021). Titratable acidity was determined by the method described by (Anihouvi, Sakyi-Dawson, Ayernor, and Hounhouigan (2007).

Statistical analysis

Data were analyzed using Statistica (version 7.1, StatSoft France, 2004), and significance was accepted at probability $p < .05$ with one-way analysis of variance (ANOVA) using least significant difference method of Fisher.

Results and discussion

Microbiological quality of marinated and fermented fish flesh

Effect of the marinating on the fish flesh

Table 1 presents microbiological characteristics of the fresh fish flesh and marinated fish flesh. The marinating reduces the total viable count (TVC) of the fresh fish flesh from 5.4 Log CFU/g to 4.7 Log CFU/g. *Enterobacteriaceae*, considered as fecal contamination indicator, were reduced from 2.5 Log CFU/g to less than 1 Log CFU/g. *Staphylococcus aureus* was lower than 1 Log CFU/g in the fresh fish flesh and marinated fish flesh. So, *Enterobacteriaceae* counts

Table 1. Microbiological characteristics of fresh fish after marinating.

Parameters (Log (CFU/g)	Fresh fish flesh	Marinated fish flesh
Total Viable Count (TVC)	5.36 ± 1.7[a]	4.70 ± 0.17[a]
Lactic acid bacteria (LAB)	ND	4.93 ± 1.37
Coagulase negative *Staphylococcus* (CNS)	ND	2.55 ± 0.12
Staphylococcus aureus and coagulase positive *Staphylococcus* (*CPS*)	< 1[a]	< 1[a]
Enterobacteriaceae	2.5 ± 0.2[b]	< 1[a]

[a]: Means with different letters in row are significantly different ($p < 0.05$); ND: Not Determined

were significantly ($p < 0.05$) reduced by the marinating process. Sallam (2008) and Ezeama (2012) reported that the use of citric acid, salt and spices have bacteriostatic and bactericide effects on the flora.

Changes in total viable count during the fermentation

The change in total viable count during the fermentation of marinated cassava fish flesh is presented in Figure 2. TVC of the inoculated fish products increased gradually from 6.7 Log CFU/g to 8.2 Log CFU/g after 36 hours of fermentation, while TVC of the control samples remained stable after the same fermentation period. A significant difference ($p < .05$) was observed between the TVC of inoculated product and that of the control product from 6 hours to 36 hours of fermentation. This was due to the increase of added inoculums and shows the aptitude of both strains used to be developed in the substrate such as fish flesh.

Changes in Lactic acid bacteria counts during fermentation

Lactic acid bacteria (LAB) populations in the marinated fish flesh used as control decreased from 4.9 to 2.8 Log CFU/g during 36 hours of fermentation but their numbers in the inoculated product increased progressively from 6.20 to 8.1 Log CFU/g during the same fermentation times (Figure 3). LAB counts of inoculated products were significantly ($p < .05$) higher than that of control samples. This significant difference was due to the growth of the *Lactobacillus*

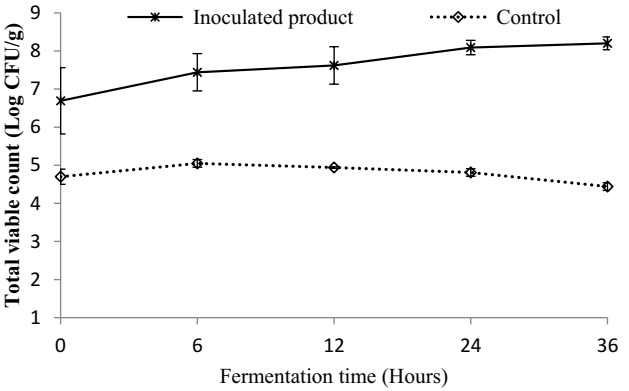

Figure 2. Changes in Total Viable Count (TVC) of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

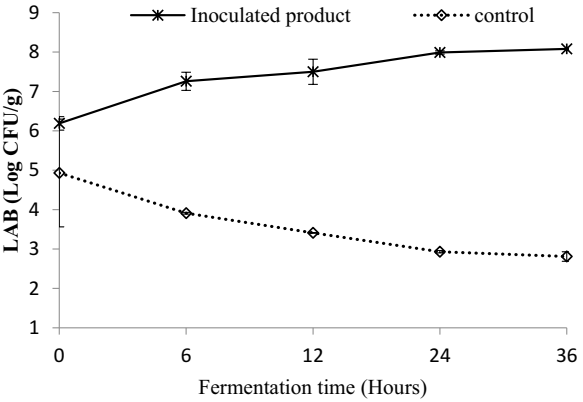

Figure 3. Changes in Lactic Acid Bacteria (LAB) of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

plantarum strain inoculated. The increase in LAB count in the inoculated product was due their capability to use the substrates of the fish flesh environment to produce metabolites (e.g lactic and acetic acids) and promote their own growth (Van, Foo, H. L., Loh, & Bejo, 2011). In addition, the presence of garlic in the medium can also stimulate the growth of LAB (Paludan-Müller, 1999).

Changes in Coagulase negative *Staphylococcus* (CNS) counts

Figure 4 shows the gradual growth of CNS during fermentation. The CNS in the control samples varied from 2.6 to 4.9 Log CFU/g after 36 hours of fermentation but high increase in CNS counts was observed in the inoculated products with *Staphylococcus xylosus* counts varying from 6.1 to 7.3 Log CFU/g. The CNS counts in the inoculated products were significantly ($p < .05$) higher than that in the control samples. The increase of CNS in inoculated products has been reported by Kaban and Kaya (2009) during the controlled fermentation of "sucuk," a beef sausage.

Changes in undesirable viable cells during fermentation

Enterobacteriaceae, Staphylococcus aureus and coagulase positive *Staphylococcus* (CPS) counts enumerated in all samples were lower than 1 Log CFU/g. This low level of undesirable bacteria enumerated could be attributed to good hygienic practices observed during handling. Also the bactericidal effect of spices used during marinating could impair the development these pathogens. Beside its taste contribution in food, garlic (*Allium sativum*) has also a bactericidal effect, against undesirable flora such as *Staphylococcus aureus* and *Pseudomonas* due to allicin (Mah, Kim, & Hwang, 2009; Paludan-Müller, 1999). Furthermore the low level of these pathogenic bacteria could be explained by the inhibitory effect of different

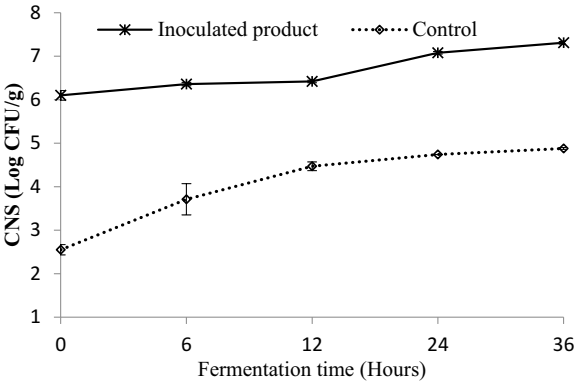

Figure 4. Changes in Coagulase Negative Staphylococci (CNS) of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

metabolites produced by both strains inoculated in the product. These results are similar to those reported by Kročko, Čanigová, Vančíková, Flimelová, and Bobková (2013) who observed the reduction of yeasts, molds and *Enterobacteriaceae* using a mixed starter cultures containing strains of *Staphylococcus xylosus, Pediococcus pentosaceus* and *Lactobacillus plantarum* for the fermentation of beef sausage.

Physico-chemical changes during the fermentation trials

Changes in pH

Figure 5 shows the changes in pH of the fish flesh inoculated with *Lactobacillus plantarum* and *Staphylococcus xylosus* strains and fish flesh without inoculum during fermentation. The pH of the inoculated samples and that of the control samples increased significantly ($p < .05$) from 5.08 to 5.20 and from 5.08 to 5.88, respectively. The pH values of the inoculated samples were significantly ($p < .05$) lower than those of the control samples from 6 hours to 36 hours of fermentation. This increase in pH observed in samples was due to the microorganisms activities. Indeed, *Staphylococcus xylosus* and other CNS present in the product had high proteolytic activity, which contributed to the development of aroma products. Stavropoulou et al. (2018) reported that addition of *Staphylococcus xylosus* in meat fermentation affected the volatile profile with lower pH of 5.3. This proteolytic activity caused pH increase due to the accumulation of nitrogenous compounds such as ammonia (Fernandes, 2009). However, the pH (5.20) of the inoculated product is in agreement with the recommended pH for meat products (pH < 6) (FCD, 2009).

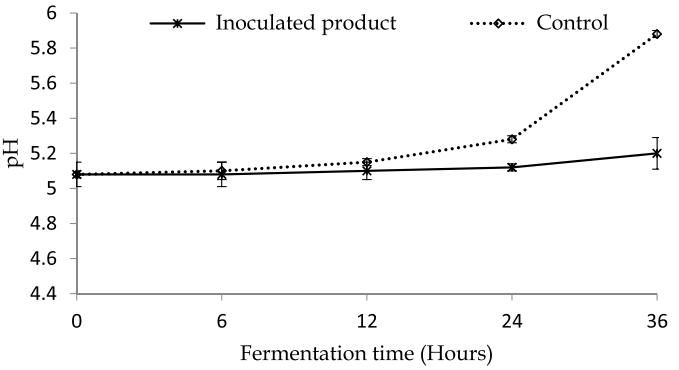

Figure 5. Changes in pH of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

Changes in total acidity during fermentation

Total acidity was determined as titratable acid equivalent to lactic acid. Titratable acid (Figure 6) increased from 0.67 to 1.23 g of lactic acid/ 100 g in inoculated samples during the fermentation time. But it decreased from 0.67 to 0.53 g of lactic acid/100 g in control samples during the same fermentation time. The increase of total acidity in inoculated samples could be attributed to the metabolic activities concomitant to the growth of lactic acid bacteria specially *Lactobacillus plantarum* within the same fermentation period (Anihouvi et al., 2012; Annan, Poll, Sefa-Dedeh, Plahar, & Jakobsen, 2003; Houngbédji et al., 2021).

Changes in total volatile nitrogen (TVN) during the fermentation

A significant ($p < .05$) increase was recorded for TVN values from 45.1 to 99.3 mg N/100 g in inoculated samples but in the control samples, it was relatively stable during the 36 hours of fermentation (45.1–50.4 mg N/100 g)

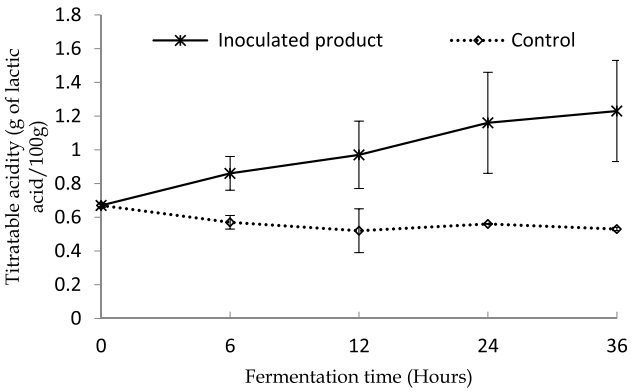

Figure 6. Changes in titratable acidity of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

(Figure 7). The high values of TVN measured in inoculated products were due to the activity of endogenous fish flora and to the proteolytic activities of the inoculated strains of *S. xylosus* as reported by (Anihouvi et al., 2012; Tremonte et al., 2010). The same trend of TVN content was reported during the fermentation of *Clarias buthupogon* for the production of fermented fish (Ezeama, 2012). This proteolytic activity was desirable for flavor development during the fermentation (Kaban & Kaya, 2009; Tremonte et al., 2010). The use of lactic acid bacteria alone to ferment fish suppressed the growth of spoilage bacteria and pathogens, but substantially inhibited the accumulation of TVN (Yin, Pan, & Jiang, 2002). So, the combined effect of both strains used as starter cultures can allow to guarantee the safety of the product and get a product with a pleasant flavor.

Characteristics of final dried product and storage product

After drying, the total viable count (TVC) of the inoculated samples (6.1 Log CFU/g) was significantly ($p < 0.05$) different with that of control samples (2.6 Log CFU/g) (Table 2). Also the LAB and CNS counts of inoculated samples were significantly ($p < 0.05$) higher than those of the control sample. TVC of the inoculated samples were in accordance with standards and the ratio TVC/LAB after drying is less than 100 (defined by the Federation of Trade and Distribution Companies in FCD) (FCD, 2009). No pathogens were detected in the inoculated samples; and yeast and mold counts were less than 1 Log CFU/g for all samples. After 90 days of storage at 30°C, no significant difference ($p > 0.05$) was recorded (Figure 8).

The water activity (a_w) and the moisture content of final dried products (inoculated ones and the control) were very low, (Table 3). The a_w of both final dried products was 0.50 which was lower than that of the whole Lanhouin

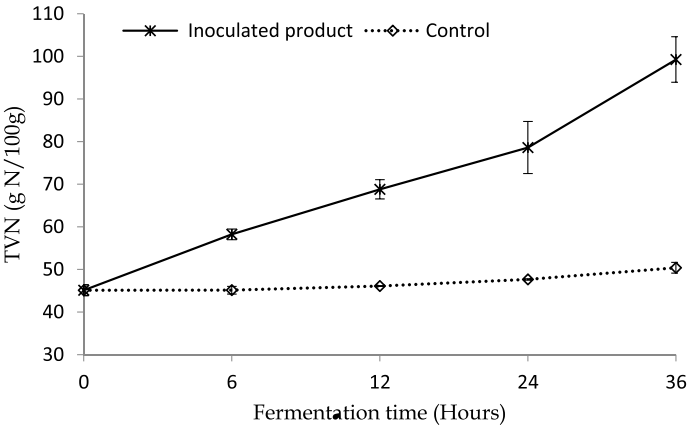

Figure 7. Changes in total volatile nitrogen (TVN) of marinated fish flesh (control) and marinated and inoculated fish flesh samples during the fermentation.

Table 2. Microbiological characteristics of final dried product.

Parameters (Log CFU/g)	Inoculated Lanhouin powder	Control
Total viable count (TVC)	6.1 ± 0.8[b]	2.7 ± 0.2[a]
Lactic acid bacteria (LAB)	6.2 ± 0.2[b]	<1[a]
Coagulase negative *Staphylococcus* (CNS)	5.4 ± 0.3[b]	2.5 ± 0.4[a]
TVC/LAB (%)	99,4	

[a]: Means with different letters in each row are significantly different ($p < 0.05$)

(0.74) reported by Kindossi et al. (2016a). The a_w of the inoculated and the control samples was lower enough to control microbial and enzymatic activity during the storage. The pH value of control sample (5.43) was significantly different from that of inoculated sample (4.70). The titratable acidity of the inoculated sample (3.7 g of lactic acid/100 g DM) was significantly ($p < 0.05$) higher than that of the control sample (2.7 g of lactic acid/100 g DM). TVN contents of inoculated samples (83.9 mg N/100 g DM) were significantly ($p <$

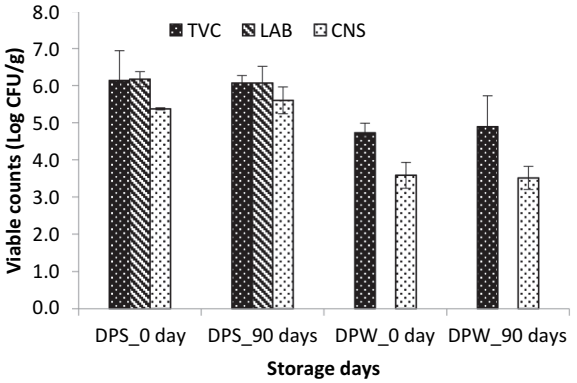

Figure 8. Microbiological status of Lanhouin powder obtained from two fermentation trials and stored during 90 days. DPS_0: final dried product obtained after oven drying of ground fish flesh fermented with starter (0 day of storage)DPS_90: final dried product obtained after oven-drying of ground fish flesh fermented with starter (90 days of storage)DPW_0: final dried product obtained after oven-drying of ground fish flesh fermented without starter (0 day of storage)DPW_90: final dried product obtained after oven-drying of ground fish flesh fermented without starter at 90 day of storage

Table 3. Physico-chemical characteristics of final dried products.

Parameters	Inoculated Lanhouin powder	Control
a_w	0.50 ± 0.00[a]	0.50 ± 0.01[a]
Moisture (g/100 g)	7.5 ± 0.1[a]	7.5 ± 0.0[a]
pH	4.70 ± 0.10[a]	5.43 ± 0.12[b]
Total acidity (g lactic acid/100 dry matter)	3.7 ± 0.1[b]	2.7 ± 0.0[a]
TVN (mg N/100 g dry matter)	83.9 ± 7.7[b]	70.3 ± 4.4[a]
Histamine (mg/100 g dry matter)	< 0.1[a]	< 0.1[a]
Cadaverine (mg/100 g dry matter)	0.3 ± 0.4[a]	< 0.1[a]
Putrescine (mg/100 g dry matter)	< 0.1[a]	< 0.1[a]
Spermidine (mg/100 g dry matter)	4.3 ± 4.4[a]	4.1 ± 4.0[a]

[a]: Means with different letters in each row are significantly different ($p < 0.05$);
0.1 mg/100 g: detected biogenic amine level

Table 4. Changes in physico-chemical characteristics of final dried product during the storage.

Parameters	DPS_0 day	DPS_90 days	DPW_0 day	DPW_90 days
a_w	0.50 ± 0.00[a]	0.50 ± 0.00[a]	0.50 ± 0.01[a]	0.50 ± 0.00[a]
Moisture (g/100 g)	7.5 ± 0.1[a]	7.7 ± 0.2[a]	7.5 ± 0.0[a]	7.7 ± 0.2[a]
pH	4.70 ± 0.10[a]	4.73 ± 0.06[a]	5.43 ± 0.12[b]	5.37 ± 0.06[b]
Total acidity (g lactic acid/100 g dry matter)	3.7 ± 0.1[a]	3.9 ± 0.3[a]	2.7 ± 0.0[b]	2.9 ± 0.2[b]
TVN (mg N/100 g dry matter)	83.9 ± 7.7[b]	78.3 ± 7.7[ab]	70.3 ± 4.4[a]	73.1 ± 4.4[ab]
Histamine (mg/100 g dry matter)	<0.1[a]	<0.1[a]	<0.1[a]	<0.1[a]
Cadaverine (mg/100 g dry matter)	0.3 ± 0.4[a]	7.68 ± 0.84[b]	<0.1[a]	2.6 ± 1.6[a]
Putrescine (mg/100 g dry matter)	<0.1[a]	3.2 ± 0.7[b]	<0.1[a]	0.9 ± 0.6[a]
Spermidine (mg/100 g dry matter)	4.3 ± 4.4[a]	40.2 ± 6.2[a]	4.1 ± 4.0[a]	21.6 ± 24.7[a]

[a]: Means with different letters in each row are significantly different ($p < 0.05$);
DPS_0: final dried product obtained after oven drying of ground fish flesh fermented with starter (0 day of storage)
DPS_90: final dried product obtained after oven drying of ground fish flesh fermented with starter (90 days of storage)
DPW_0: final dried product obtained after oven drying of ground fish flesh fermented without starter (0 day of storage)
DPW_90: final dried product obtained after oven drying of ground fish flesh fermented without starter at 90 day of storage

0.05) higher than that of control (70.3 g N/100 g DM). Histamine was not detected in both inoculated and control samples. Other biogenic amines such as cadaverine and spermidine were detected at very low levels showing that the final dried product was safe.

No significant differences ($p > 0.05$) for a_w, moisture, pH, titratable acidity and TVN were observed for each inoculated Lanhouin powder from 0 to 90 days of storage. But after 90 days of storage, cadaverine, putrescine and spermidine levels were significantly ($p < 0.05$) higher for Lanhouin powder obtained with starter (Table 4). These increased levels mainly of spermidine could be attributed to the presence of starter culture. These observations are in agreement with Martuscelli, Crudele, Gardini, and Suzzi (2000) who reported that seven *Staphylococcus xylosus* strains tested in their study had a potential to produce biogenic amines mainly spermidine and spermine but most of *Staphylococcus xylosus* strains study had a remarkably high potential to reduce the level of histamine in traditional fermented sausage. *Staphylococcus xylosus* and *Lactobacillus plantarum* were also found as protective culture due to the fact that they produced bacteriocin-like inhibitory substances and have the highest antimicrobial activity against pathogens microorganisms defined as amine producers (Mah & Hwang, 2009b; Tosukhowong et al., 2011).

Conclusion

The results of this study showed the good aptitude of two strains of *Staphylococcus xylosus* and *Lactobacillus plantarum* to be used as bio-preservatives to control the production of fish-based flavor enhancer condiment which could be used as a new type of Lanhouin for new markets. The two strains were able to standardize fermentation when it is well applied to the right proportion. Moreover, the fermentation time has been reduced. Indeed, during

the traditional processing, fermentation was carried out for 72–216 hours (in tank, can, jar, basket) or 15–29 days (buried in ground) while in this improved process, fermentation was done in 36 hours. Satisfactory fermentation conditions with lower pH values, lower biogenic amines level and absence of pathogenic bacteria were obtained with respect to the control where the fermentation conditions were spontaneous. Regarding, the final dried product obtained by bio-preservation and stored at ambient temperature (30 ± 2°C) during 90 days, the levels of LAB and CNS were 6.2 Log CFU/g and 5.4 Log CFU/g, respectively, and with a ratio of TVC/LAB < 100. This means that the bio-preservation guarantees the harmlessness and improves the shelf-life of the Lanhouin powder. However, further works need to be done for better understanding of bio-preservation processes of fish. This could provide inclusive knowledge on the processing which will help for the development of a new flavor enhancer condiment.

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