academicJournals

Vol. 10(10) pp. 227-237, October 2016
DOI: 10.5897/AJFS2016.1443
Article Number: 6735A6259908
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Microbial population and physico-chemical composition of an African Fish based flavouring agent and taste enhancer

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Received 24 March, 2016; Accepted 30 June, 2016

Sixty samples of a traditional flavouring agent and taste enhancer (FATE) locally referred to as Lanhouin obtained by spontaneous fermentation of cassava fish (*Pseudotolithus* sp.) and king fish (*Scomberomorus tritor*), used as traditional condiment to enhance the flavour of many dishes were purchased from processing sites and markets, for physico-chemical and microbiological characterization using standard methods. FATE samples exhibited similar water activity level (0.75-0.77), variable pH values (6.88-7.68), variable amounts of dry matter (43.4-47.2 g/100 g), salt (18.7-26.6 g/100 g DM), protein (49.2–53.8 g/100 g DM), lipid (10.8-47.4 g/100 g DM), thiobarbituric acid reactive substances (24.8 to 27.1 mg malonaldehyde/kg DM), total volatile nitrogen (453.6 to 618.6 mg N/100 g DM) and acidity index (1.7 to 4.9 g oleic acid /100 g DM), various organic acids and histamine contents within acceptable limit of 20 mg/100 g for 87% of samples analysed. For all these chemical components, significant differences ($p < 0.05$) were observed between fish species and between sampling places. Total viable counts were ranged between 3.6 to 4.2 Log cfu/g. No *Salmonella* and *Listeria monocytogenes* were found in any FATE sample. The technological flora such as lactic acid bacteria were enumerated (1.2 Log cfu/g) in 42% of samples while coagulase negative *Staphylococci* were found in all the FATE samples (2.9-3.9 Log cfu/g).

Key words: King fish, cassava fish, flavouring agent, *Lanhouin*, fermentation, quality characteristics.

INTRODUCTION

Lanhouin, a traditional fermented fish-based condiment is processed in the coastal areas of West African countries

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including Benin, Togo, Ghana, Nigeria and Côte-d'Ivoire. It is mostly used as taste enhancer and flavouring agent in many types of dishes (Anihouvi et al., 2005; Kindossi et al., 2012). The production of Lanhouin is essentially based on endogenous knowledge, laborious and time consuming. The raw materials used for Lanhouin production include the fish and the salt, and the fermentation is spontaneous and uncontrolled (Anihouvi et al., 2012). Different processes and different types of fish are used to produce Lanhouin, but the end-product seems apparently the same. For the production, the fresh fish is scaled, gutted, washed and left for ripening during 8 to 11 h before the maturated fish is treated with salt and allowed to ferment for 3 to 9 days. So, the various technologies applied are still artisanal, and consequently the quality of the final product is unpredictable. In addition, the conditions of production are not likely to guarantee its harmlessness. Moreover, the most significant operations such as ripening and fermentation are not well defined, nor controlled whereas they determine the final quality of Lanhouin (Anihouvi et al., 2005; Kindossi et al., 2012). In order to improve the process and quality of Lanhouin, it would be necessary to characterise this product on both microbiological and physico-chemical aspects. The current investigation aims to assess the quality of Lanhouin obtained from two types of fish mainly used for its commercial production.

MATERIALS AND METHODS

Sample source and sampling

A total of 60 samples of Lanhouin made with cassava fish (*Pseudotolithus sp.)* and king fish/spanish mackerel (*Scomberomorus tritor)* were randomly collected in sterile stomacher bags from 12 retailers in market at Comé and Djodah cities and from nine (09) processing sites in Grand-Popo municipality in the southern region of Benin. The Lanhouin samples were transported to the laboratory in an ice box filled with dry ice and maintained at 4°C. The microbiological analyses were performed within 24 h. The remaining samples were kept at 20°C for physico-chemical and biochemical analyses.

Microbiological analyses

Ten (10) g of each Lanhouin 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 homogenised for two min, using a Stomacher (Lab-Blender, Model 80, Seward Medical, London, UK) (1999). One ml of the suspension was serially used for microbial counts according to ISO norms.

Total viable counts (TVC), Lactic Acid Bacteria (LAB) and *Enterobacteriaceae* were enumerated using Plate Count Agar (PCA, Oxoid CM0325, Basingstoke, Hampshire, England), de Man, Rogosa, Sharpe agar (MRS, Oxoid CM0361, Basingstoke, Hampshire, England) and Violet Red Bile Glucose Agar (VRBG, Oxoid, CM0485, Basingstoke, Hampshire, England) respectively. Yeasts and moulds 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 1988). PCA (ISO-4833 2003) and MRS (ISO-15214 1998) plates were incubated at 30°C for 72 h. *Enterobacteriaceae* plates were incubated at 37°C for 24 h (ISO-21528 2004). *Escherichia coli, Bacillus cereus, Clostridium perfringens* and *Staphylococcus aureus* were enumerated according to ISO methods using Tryptone bile glucuronide (TBX, CM0945, Basingstoke, Hampshire, England), Bacillus cereus agar base (Oxoid, CM0617, Basingstoke, Hampshire, England), TSC & SFP (Oxoid CM0587, Basingstoke, Hampshire, England) supplemented with egg yolk emulsion (SR0047, Basingstoke, Hampshire, England) and TSC supplement (SR0088, Basingstoke, Hampshire, England), and 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 44°C for 24 h (ISO-16649 2001), 30°C for 48 h (ISO-7932 2004) and 37°C for 24 h (ISO-6888 1999; ISO-7937 2004) respectively.

Salmonella were investigated on Xylose-Lysine-Desoxycholate Agar (Oxoid CM0469, Basingstoke, Hampshire, England) after pre-enrichment of 25 g of sample in buffered peptone (Oxoid CM 509 Basingstoke, Hampshire, England) and selective enrichment in Rappaport-Vassiliadis Broth (Oxoid CM 669 Basingstoke, Hampshire, England) and Muller-Kauffmann Tetrathionate Novobiocin broth (MkTTn, Oxoid CM 1048, Basingstoke, Hampshire, England) (ISO-6579 2002).

Listeria monocytogenes were examined on Palcam Agar Base (Oxoid CM0617, Basingstoke, Hampshire, England) and Chromogenic Listeria Agar Base (Oxoid CM1084 Basingstoke, Hampshire, England) after pre-enrichment of 25 g of sample in Fraser Broth Base (Oxoid CM 0895 Basingstoke, Hampshire, England) and Listeria Enrichment Broth Base (Oxoid CM 0863 Basingstoke, Hampshire, England) (ISO-11290 2004).

Determination of physico-chemical characteristics

pH of samples was measured with a pH meter (Hanna Instrument HI 9318) according to reference method (ISO-2917 1999). Water activity (aw) was measured with a thermo-hygrometer recorder C056696 (Rotronic Hygrolab 2, 8303 Bassersdorf) according to the method described by Anihouvi et al. (2006). Protein content was determined according to reference method (ISO-937 1978). Total volatile nitrogen (TVN) was estimated using perchloric acid extraction and steam distillation method (Ababouch, 1995). Lipid was determined according to Folch method (Folch et al., 1957). Thiobarbituric acid reactive substances (TBARS) and Acid index were determined according to Pearson (1976) and AFNOR (1993), method NF T. 60-204. Sodium chloride content (NaCl) was determined by measuring the chloride ion concentration with a chloride analyser (Corning MKII model 926, Sherwood Scientific Ltd, UK) after extraction in 0.3 N nitric acid.

Determination of organic acids

Samples preparation

Approximately 150 mg of each Lanhouin sample were suspended in 1 ml of 5 mM H_2SO_4 and mixed thoroughly for 30 min using vortex mixture. After centrifugation of the food suspension at 4140 rpm for 5 min, the supernatant was collected and filtrated through a 0.45 µm microporous membrane before the determination of

organic acid contents (Mestres et al., 2004).

HPLC equipment

Organic acids were determined using an HPLC (Knauer system, Germany) equipped with a Rheodyne 7125 injector, an on-line solvent degasser with LPG Smartline manager 5050 (ADA110606103, Knauer, Germany), Smartline RI Detector 2300 (n°110542, Knauer, Germany) and Spectra system UV2000, a Knauer system controller Smartline, a pump 1000 (n°111235, Knauer, Germany), a Supelcogel H 59304-U Column (30 cm x 7.8 mm ID, Bellefonte, PA, USA) with Supelguard C610H pre-column (5 cm x 4.6 mm ID) and a 20 µl injector loop (Rheodyne, Cotati, CA, USA).

Chromatographic conditions

The analysis was carried out isocratically at a flow rate of 0.6 ml/min., employing as mobile phase water adjusted to pH 2.1 with metaphosphoric acid. The column was thermostated at 30°C. Injection volume was 20 µl and organic acids were detected at 210 nm. Citric, malic, lactic, formic, acetic, propionic acids were identified by retention and spectral data (Mestres et al., 2004).

Determination of biogenic amines

Samples preparation

Approximately 50 mg of each Lanhouin sample were suspended in 3 ml of 0.4 M perchloric acid solution, shaken for 15 min and centrifuged at 2500 × g for 20 min at room temperature. The supernatant was collected and filtrated through Whatman paper n°1.

Derivatisation of sample extracts

Two hundred and fifty (250) µl aliquot of each sample extract was mixed with 50 µl of 2 M sodium hydroxide and 75 µl saturated sodium bicarbonate (Na2CO3). Five hundred (500) µl of a dansyl chloride (Dns-Cl) solution was prepared by ultrasonic dissolution of 5 mg Dns-Cl (1-dimethylamino naphthalene-5-sulfonyl chloride, Sigma Aldrich) per 1 ml acetone (Fisher Scientific) and the mixture was then thoroughly vortexed for 1 min. For derivatisation, the mixture was incubated in water bath at 60°C for 45 min in the dark. After this incubation period, 25µl of 25% ammonium hydroxide was added to remove the excess of dansyl chloride and the mixture was again incubated for 30 min at room temperature in the dark. After this second incubation period, 375 µl of acetonitrile was added to the mixture. Finally, the mixture was filtered through 0.45 µm pore-size filter (Millipore Co., Bedford, MA) and injected into the chromatographic column (Mah et al., 2002; Zaman et al., 2010).

Chromatographic conditions

The same HPLC unit described above was used for biogenic amines determination. Chromatographic separation of biogenic amines was carried out according to the procedure developed by Mah et al. (2002) with minor modifications. The analysis was performed using Column Kromasil 100-5C18 (250 x 4.6 mm E72991) (Rheodyne, Cotati, CA, USA) with water (solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 0.8 ml/min. The program was set for a linear gradient starting from 50% of solvent B to reach 90% of the solvent at 19 min. The sample volume injected was 20 µl and the sample was monitored at 254 nm. Histamine, cadaverine, putrescine and spermidine were identified by retention and spectral data.

Statistical analysis

Data were analysed using Statistica (version 6, StatSoft France, 2004) and significance was accepted at probability $p < 0.05$ with one-way analysis of variance (ANOVA) by using least significant difference method of Fisher. Correlations between variables were evaluated using principal component analysis with XLSTAT software (version 2011, Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Physico-chemical characteristics of Lanhouin samples

The results of physico-chemical analyses of Lanhouin samples are summarized in Table 1. The dry matter content of all samples varied from 43.9 to 47.2 g/100 g for cassava fish and from 43.4 to 45.4 g/100 g for king fish Lanhouin samples. These values agree with those previously reported for Lanhouin samples (49.9 and 43.4 g/100 g from cassava fish and king fish respectively) (Anihouvi et al., 2006), Momoni samples (44.60-48.58 g/100 g) (Sanni et al., 2002; Essuman, 1992) and salted anchovy samples (45.84 g/100 g) (Hernández-Herrero et al., 1999). The dry matter content of market Lanhouin samples processed with cassava fish was significantly higher ($p < 0.05$) than that collected from processing sites. These variations in dry matter contents could be the result of variable drying conditions, duration between processing time and sampling time, level of salt and type of salt used during the processing (Anihouvi et al., 2006). However, the moisture content seems to be an inexact indicator of the susceptibility of a product to undergo microbial spoilage. A key factor, which determines the microbial stability of foods, is water activity (aw) (Anihouvi et al., 2006).

The water activity (aw) of all Lanhouin samples varied from 0.74 to 0.75 and 0.75 to 0.77 for cassava fish and king fish Lanhouin samples respectively, which were slightly above 0.70 as reported by Anihouvi et al. (2006). These values are too high to prevent enzymatic activity and microbial proliferation including food poisoning bacteria during storage. These values of aw classed Lanhouin samples as intermediate moisture content product (Maltini et al., 2003).

pH values of samples ranged between 7.11 and 7.40 and between 6.88 and 7.68 for cassava fish and king fish Lanhouin samples respectively. Whatever the species of fish, the pH of samples collected from the processing sites was significantly ($p < 0.05$) lower than that of the

Table 1. Physico-chemical characteristics of *Lanhouin* samples collected from markets and processing sites (Means ± Standard Deviation).

Parameter	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Water activity (Aw)	0.74±0.02[a]	0.75±0.02[a]	0.75±0.03[a]	0.77±0.02[a]
Dry matter (g/100 g)	43.9±2.1[b]	47.2±2.4[a]	43.4±1.9[a]	45.4±2.8[a]
pH	7.11±0.34[a]	7.40±0.24[b]	6.88±0.72[a]	7.68±0.19[b]
NaCl (g/100 g. DM)	26.6±6.6[b]	19.3±5.0[a]	23.5±12.1[a]	18.7±7.6[a]
Protein (g/100 g DM)	53.8±7.6[a]	49.3±8.1[a]	52.6±4.9[a]	49.2±6.8[a]
Lipid (g/100 g DM)	12.2±7.0[a]	10.8±7.4[a]	42.8±17.0[a]	47.4±21.1[a]
TBARS (mg malonaldehyde / kg DM)	27.1±3.2[a]	25.5±1.9[a]	26.7±3.7[a]	24.8±2.7[a]
TVN (mg N / 100 g DM)	453.6±125.3[a]	457.9±132.4[a]	553.6±94.5[a]	618.6±174.8[a]
Acidity index(% oleic acid DM)	1.8±0.4[a]	1.7±1.1[a]	4.3±1.0[a]	4.9±1.4[a]

[a,b]: Means with different letters according to each row and each species of fish are significantly different ($p<0.05$); n: number of samples analysed; DM : dry matter.

samples collected at market level. This could be due to the fact that the samples collected from processing sites were freshly made while the ones collected at market level were probably old. Previous studies on Lanhouin and Momone, a Lanhouin like-product have shown that a potential problem of these products is the continuous bacterial and enzymatic activity after processing, leading to the rise of pH values during storage (Anihouvi et al., 2006). These pH values recorded agree with those reported on Lanhouin samples from cassava fish (7.3) and king fish (7.6) (Anihouvi et al., 2006). The pH values of both fish Lanhouin were slightly higher than those of Momoni (6.47-6.56) (Sanni et al., 2002) and Adjuevan (5.20-6.10) (Koffi-Nevry et al., 2011).

The salt contents of Lanhouin samples varied from 19.3 to 26.6 g/100g DM for cassava fish Lanhouin and 18.7 to 23.5 g/100 g DM for king fish Lanhouin. The salt content of cassava fish Lanhouin collected at market level was significantly lower ($p<0.05$) than that of cassava fish Lanhouin collected from processing sites. But for king fish, no significant difference was observed between the salt content of Lanhouin samples collected from processing sites and that of Lanhouin samples collected at market level. The variations in salt contents could be attributed to the fact that the amount of salt used during processing is not standardized, so varied from one processor to another (Kindossi et al., 2012). The values of salt content were slightly higher than those reported during a previous study (14.63 g/100 g DM for cassava fish and 10.42 g/100 g DM for king fish Lanhouin) collected in the Atlantic municipalities of Benin (Anihouvi et al., 2006); but these values agree with the salt contents of 25 g/100 g DM and 20.1 g/100 g DM obtained on the laboratory samples of Lanhouin made with cassava fish and king fish respectively (Dossou-Yovo et al., 2011).

The protein contents varied from 49.3 to 53.8 g/100 g DM and 49.2-52.6 g/100 g DM in all Lanhouin samples prepared with cassava fish and king fish respectively. For both fish, there was no significant difference ($p>0.05$) between the protein content of market Lanhouin and that of Lanhouin collected from processing sites. The protein values obtained for all samples agree with those reported in Lanhouin samples obtained with cassava fish (46.9–59.3 g/100 g DM) and king fish (48.2 to 68.2 g/100 g DM) (Anihouvi et al., 2006) and in Momoni in general (36.0-49.1 g/100 g DM) (Sanni et al., 2002) and Momoni samples made with king fish (49.0 to 57.7 g/100 g DM) (Nketsia-Tabiri and Sefa-Dedeh, 2000).

The lipid contents varied from 10.8 to 12.2 g/100 g DM and 42.8 to 47.4 g/100 g DM for cassava fish Lanhouin and king fish Lanhouin samples respectively. For each species of fish there was no significant difference ($p>0.05$) between the lipid contents of all Lanhouin samples collected from market and from processing sites. But the lipid contents of king fish Lanhouin were significantly ($p<0.05$) higher than those of cassava fish Lanhouin. The difference in lipid contents of Lanhouin samples prepared with the two species of fish is due to the fact that king fish is a fatty fish while cassava fish is a lean fish (Huss, 1988; Love, 1997).

Thiobarbituric acid (TBARS) contents ranged between 25.5 and 27.1 mg malonaldehyde/kg DM for cassava fish Lanhouin, and between 24.8 and 26.7 mg malonaldehyde/kg DM for king fish Lanhouin samples. No significant difference ($p>0.05$) was observed in the thiobarbituric acid contents for all Lanhouin samples whatever the species of fish used. Thiobarbituric acid numbers provides an indication of onset of lipid oxidation (Hernández-Herrero et al., 1999). The current values of thiobarbituric acid recorded on Lanhouin samples were higher than values of 10.6 to 12.2 mg malonaldehyde/kg

Table 2. Organic acids composition of *Lanhouin* samples collected from markets and processing sites (Means±Standard deviation).

Organic acids (mg/g DM)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Lactic acid	2.9±2.8[a]	1.5±1.9[a]	4.3±1.5[a]	3.5±0.9[a]
Citric acid	1.9±2.3[a]	1.9±2.0[a]	1.3±1.7[a]	0.6±0.9[a]
Malic acid	1.1±2.1[a]	1.2±1.9[a]	0.5±1.2[a]	0.4±0.7[a]
Formic acid	1.2±1.6[a]	0.9±1.9[a]	0.5±0.5[a]	1.0±1.8[a]
Acetic acid	5.6±1.4[a]	4.7±2.2[a]	4.6±1.2[a]	4.6±1.7[a]
Propionic acid	1.3±1.8[a]	1.1±1.4[a]	3.3±4.8[a]	2.4±1.4[a]

[a,b,c]: Means with different letters according to each row and each species of fish are significantly different ($p<0.05$); n: number of samples analysed; DM: dry matter.

for cassava fish Lanhouin, and 16.6 to 21.7 mg malonaldehyde/kg DM for king fish Lanhouin reported by Anihouvi et al. (2006). However, lower values in TBARS of 6.9-7.4 mg malonaldehyde/kg DM have been reported by Oduor-Odote and Obiero (2009) on smoked fish. The total volatile nitrogen (TVN) contents of samples varied from 453.6 to 457.9 mg N/100 g DM for cassava fish Lanhouin, and 553.6 to 618.6 mg N/100 g DM for king fish Lanhouin. No significant differences ($p>0.05$) were observed for the TVN contents of all Lanhouin samples within and between species. Level of TVN in fish is generally used as spoilage indicator due to bacterial and enzymatic action, leading to proteins degradation and a low nutritional value of the end product (Anihouvi et al., 2006; Hernández-Herrero et al., 1999). These values of TVN agree with those reported by Anihouvi et al. (2006) in Lanhouin samples made with cassava fish (530.5–650.0 mg N/100 g DM) and king fish (827.6-898.2 mg N/100 g DM).

The acidity index contents in cassava fish Lanhouin (1.7 to 1.8 g oleic acid/100 g DM) were lower than those of king fish Lanhouin (4.3 to 4.9 g oleic acid/100 g DM). For each species no significant difference ($p>0.05$) was recorded for the acidity index of all Lanhouin samples. These values of acidity index were lower than those reported for a previous study on market samples of Lanhouin (Anihouvi et al., 2006).High acidity index content is an indication of microbial and enzymatic spoilage such as lipases activities (Anihouvi et al., 2006; Hernández-Herrero et al., 1999).The acceptable limit of acidity index is about 0.5-1.5 oleic acid/100 g (Saritha and Patterson, 2012). Moreover, according to Daramola et al.(2007), in most fish oils, the rancidity is noticeable when the acidity index is between 0.5-1.5 oleic acid/100 g.The organic acids composition in Lanhouin is summarized in Table 2. Levels of lactic acid varied from 1.5 to 2.9 mg/g DM and 3.5 to 4.3 mg/g DM for cassava fish Lanhouin and king fish Lanhouin respectively. No significant difference ($p>0.05$) was recorded for lactic acid contents in all Lanhouin samples collected from market and processing sites within species but significant difference ($p<0.05$) was noted between species. Lactic acid contents in Lanhouin samples analysed were lower than those reported in other salted and fermented fish products (16 mg/g DM) (Kuda et al., 2002), salted fish (48.10 mg/g DM), fish sauce (aji-no-susu) (57.14 mg/g DM) (Kuda et al., 2009). Levels of acetic acid varied from 4.7 to 5.6 mg/g DM and 4.6 mg/g DM were determined for cassava fish and king fish Lanhouin samples respectively. The levels of acetic acid contents were higher than other organic acids determined in the Lanhouin samples. These values of acetic acid are in agreement with those reported by Essuman (1992) in fermented fish from Mali. No significant difference ($p>0.05$) was recorded for acetic acid contents in all Lanhouin within and between species. Other organic acids such as citric, malic, formic and propionic acids were also detected in all Lanhouin samples, although their amounts were very low.

Various biogenic amines including histamine, putrescine, cadaverine and spermidine were detected in variable amounts in the Lanhouin samples analysed (Table 3). Histamine content in cassava fish Lanhouin varied from 10.1 to 33.0 mg/100 g. No significant difference ($p>0.05$) was observed for histamine contents in all cassava fish Lanhouin. Independently to sampling place, histamine contents less than 20 mg/100 g was obtained in 87% of Lanhouin samples made from cassava fish (Pseudotolothus sp.) while 3 and 10% of samples showed histamine levels ranging between 20-40 mg/100 g and exceeding 40 mg/100 g respectively. Regarding Lanhouin samples obtained from king fish (Scomberomorus tritor), their histamine contents varied from 23.7 to 31.2 mg/100 g. The histamine contents in king fish Lanhouin collected from market was not significantly different ($p>0.05$) with those of king fish Lanhouin collected from processing sites. According to the European Union regulation (CE n°853/2004), for fishery products which have undergone enzyme maturation treatment, on 9 samples analysed, the average histamine

Table 3. Biogenic amines determined in *Lanhouin* samples collected from markets and processing sites (mean ± standard deviation).

Biogenic amines (mg/100 g wwb)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Histamine	33.0±23.8[a]	10.1±3.4[a]	31.2±14.6[a]	23.7±13.0[a]
Cadaverine	165.1±25.0[a]	170.5±31.3[a]	323.8±141.2[a]	104.5±24.0[a]
Putrescine	33.9±6.0[a]	29.1±6.2[a]	37.2±6.3[b]	16.1±4.5[a]
Spermidine	56.5±23.1[a]	77.2±25.9[a]	47.4±8.7[a]	69.6±36.9[a]

[a,b]: Means with different letters according to each row and each species of fish are significantly different ($p<0.05$); n: number of samples analysed; wwb: wet weight basis.

Table 4. Microbial quality of *Lanhouin* samples collected from markets and processing sites (Means±Standard deviation).

Parameter (Log CFU/g)	Cassava fish		King fish	
	Processing sites (n=18)	Market (n=12)	Processing sites (n=18)	Market (n=12)
Total viable count	3.7±0.6[a]	4.0±0.7[a]	3.6±0.5[a]	4.2±0.2[a]
Enterobacteriaceae	<1[a]	1.6±1.4[b]	<1[a]	<1[a]
Escherichia coli	<1[a]	<1[a]	<1[a]	<1[a]
Bacillus cereus	1.7±0.9[b]	<1[a]	1.6±0.9[b]	<1[a]
Staphylococcus aureus and CPS	1.6±0.5[a]	1.9±0.2[a]	1.8±0.4[a]	1.5±0.3[a]
Clostridium perfringens	1.0±0.8[a]	<1[a]	<1[a]	1.4±1.3[a]
Yeasts and moulds	1.4±1.0[a]	1.7±0.9[a]	<1[a]	1.8±1.0[b]
Lactic acid bacteria (LAB)	1.0±0.9[a]	1.2±0.9[a]	1.2±0.9[a]	<1[a]
Coagulase negative *Staphylococci* (CNS)	3.5±1.3[a]	2.9±0.6[a]	3.9±1.0[b]	3.2±0.6[a]
*Listeria monocytogenes**	Absent	Absent	Absent	Absent
*Salmonella**	Absent	Absent	Absent	Absent

[a,b]: Means with different letters according to each row and each species of fish are significantly different ($p<0.05$); n: number of samples analysed; * search in 25 g sample.

content must be 20 mg/100 g or less; no more than 2 samples may have levels between 20 mg and 40 mg/100 g; and no sample may have a level above 40 mg/100 g. In this respect, 67% of king fish Lanhouin contained histamine levels less than 20 mg/100 g, 3% of them had histamine contents of 20 mg/100 g, and 13% had histamine contents ranging between 20-40 mg/100 g, while 17% showed histamine levels higher than 40 mg/100 g. Summarizing, both Lanhouin samples collected from processing sites and markets do not comply with the regulatory indicated above in terms of histamine content. These results showed that the type of fish can impair the production of histamine. Moreover, in a previous study (Anihouvi et al., 2006) where Lanhouin samples were purchased from the processors and from the markets, the histamine contents in the majority (75%) of samples, mainly the ones prepared with king fish, exceeded the recommended level of 20 mg/100 g stipulated by the Australian Food Standards Code (AFSC, 2001). Furthermore, the levels of putrescine, cadaverine and spermidine found in the Lanhouin samples during the current study were high. This may increase the toxic effect of histamine as both putrescine, and cadaverine are known to potentiate histamine toxicity (Lehane and Olley, 2000; Houicher et al., 2013; Tsai et al., 2006); also the toxicity of histamine can be increased by the presence of other biogenic amines (spermine, spermidine, dopamine and agmatine) which can have a synergistic effect (Duflos, 2009; FAO/WHO, 2012). Concerning putrescine and cadaverine no safe levels have been not yet set for human consumption (FAO/WHO, 2012).

Microbial population of Lanhouin samples

The microbial population of Lanhouin samples summarized in Table 4 revealed that the total viable counts (TVC) of these samples varied from 3.7 to 4.0 Log cfu/g and 3.6 to 4.2 Log cfu/g for all Lanhouin processed

with cassava fish and king fish respectively. Such levels of TVC were within the acceptable limit of 5 Log cfu/g (ICMSF, 2011; Fernandes, 2009). No significant difference was observed for TVC recorded in all Lanhouin samples within and between species. *Enterobacteriaceae*, considered as faecal contamination indicator were detected in few numbers (<1-1.6 Log cfu/g) in 20% of market samples obtained from both King fish and cassava fish, while *E. coli* count (Log cfu/g) was lower than 1 for all the samples. For fish and meat products, the authorised limit about *Enterobacteriaceae* is 3 Log cfu/g (ICMSF 2011). Thus, all the Lanhouin samples comply with this regulation. Similarly, low counts (Log cfu/g) of *B. cereus* ranging between less than 1 and 1.7 Log cfu/g were enumerated in 34% of samples. S. aureus and other Coagulase Positive *Staphyloccoccus* (CPS) were also detected in all the samples, and their loads varied from 1.5 to 1.9 Log cfu/g. The authorized level of *S. aureus* and CPS stipulated by European Commission for fish products is between 2 and 3 Log cfu/g (EC/n°2073 2005). In this regard, all the Lanhouin samples comply with this regulation, with 72% of Lanhouin samples having *S. aureus* and CPS loads less than 2 Log cfu/g and only 28% of Lanhouin samples with loads between 2 and 3 Log cfu/g. The relatively high level of *S. aureus* in some Lanhouin samples was probably due to the lack of good manufacturing practices and the quality of salt used, since previous studies by Anihouvi et al. (2006) showed the absence of *S. aureus* in Lanhouin and Momone, a Lanhouin-like Ghanaian fermented fish. Indeed, according to Kindossi et al. (2012), solar salt is the main type of salt used for salting in the sampling zones and solar salt is known for its poor microbiological quality (Plahar et al., 1999). According to Varnam and Evans (1991) *S. aureus* load can reach high levels of 5 Log cfu/g in products prepared under bad hygienic conditions and can cause food poisoning (ICMSF, 1986). Yeasts and moulds were enumerated in 67% of samples with loads ranging between less than 1 and 1.8 Log cfu/g, while Lactic acid bacteria (LAB) were found at a level of 2.2 Log cfu/g in 42% of samples. As expected, Coagulase Negative *Staphylococci* (CNS) was found in all Lanhouin samples (2.9-3.9 Log cfu/g). In contrast, pathogenic bacteria such as *Salmonella and L. monocytogenes* were not detected in any sample.

Correlation between physico-chemical and microbiological characteristics of Lanhouin samples

The NaCl contents of samples analysed were significantly ($p<0.05$) and negatively correlated with water activity ($r = -0.78$) and pH ($r = -0.67$). These correlations indicate that NaCl was used in order to reduce water activity (aw) and pH. These actions of NaCl retard or eliminate the growth of proteolytic bacteria during fermentation. It was reported that variable levels of salt can reduce considerably aw and decrease pH (Nout, 2001; Kose and Hall, 2011).

The protein contents of Lanhouin samples were significantly and negatively correlated with dry matter ($r = -0.72$, $p<0.05$) and *Enterobacteriaceae* ($r = -0.64$, $p<0.05$), and yeast and moulds ($r = -0.69$, $p<0.05$); and significantly and positively correlated with Coagulase Negative *Staphylococcus* (CNS) ($r = 0.68$ $p<0.05$). These correlations indicate that the reduction of protein value in the product was due to the protein degradation by the proteolytic activity of these microorganisms.

The lipid contents were significantly and positively correlated with TVN ($r = 0.80$, $p<0.05$), acidity index ($r = 0.97$; $p<0.05$) and propionic acid ($r = 0.76$; $p<0.05$). These high correlations indicate that fish lipids are highly unsaturated fatty acids (Halamíčková and Malota, 2010; Rael et al., 2004) and therefore, can be easily oxidized (Vidotti et al., 2011). Oxidation can affect the nutritional quality of the product by making the proteins and amino acids unavailable and also making the product unpalatable. These results showed that high lipid content carries away an increase in TVN and propionic acid contents of Lanhouin samples.

The lactic acid content was significantly and negatively correlated with yeast and moulds ($r = -0.72$, $p<0.05$), and *Enterobacteria* ($r = -0.79$, $p<0.05$), and positively correlated with CNS ($r = 0.71$, $p<0.05$). These higher correlations indicated that the lactic acid produced during the fermentation has an inhibitory effect on *Enterobacteria*, and yeast and moulds, but its presence promotes the development of CNS. These correlations confirmed the inhibitory effect of organic acids on pathogenic microorganisms (Rhee et al., 2011; Hall, 2002).

TBARS content was significantly and positively correlated with *B. cereus* counts ($r = 0.97$, $p<0.05$) and significantly and negatively correlated with yeasts and moulds counts ($r = -0.70$, $p<0.05$) and negatively correlated with lipid but not significantly ($r = -0.26$, $p>0.05$). These correlations indicate that lipid oxidation is not dependent up only on lipid substrate but depends also on microbial activities. The same result was observed as relation between TBARS and lipid during the processing of herring (Undeland et al., 1998; Undeland and Lingnert, 1999). Yeast and moulds reduce lipid oxidation and consequently TBARS contents in Lanhouin. Other studies showed that fungi and yeast were used to reduce lipid oxidation during the processing of fermented fish meal (Khodanazary et al., 2013; Yano et al., 2008). Histamine was significantly and positively correlated ($p<0.05$) with cadaverine ($r = 0.67$). This correlation indicates that the toxicity of histamine was influenced by the presence of cadaverine. This is in agreement with the findings of Naila et al. (2010) and Kose and Hall (2011) who reported that the toxic effect of histamine is increased by the presence of other biogenic amines such

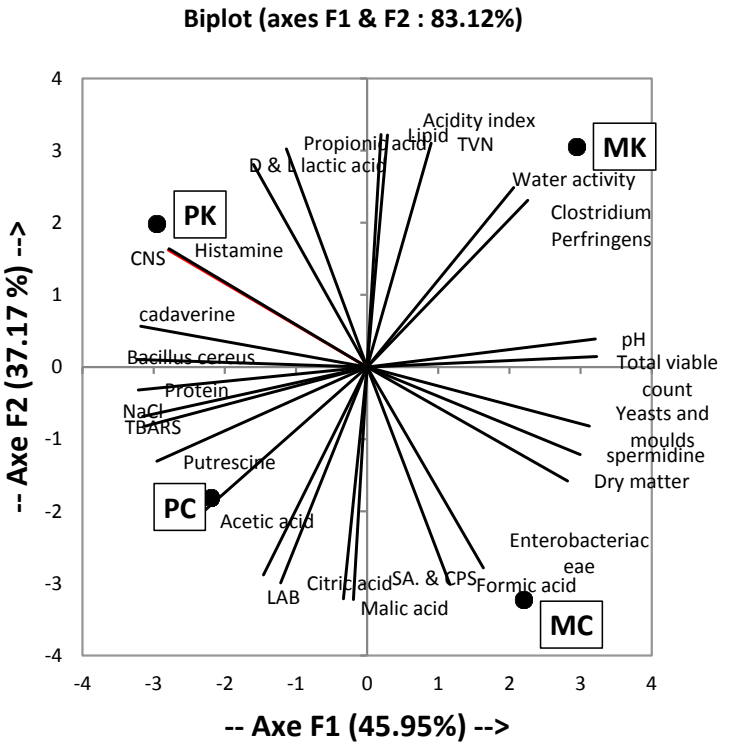

Figure 1. Principal component analysis on physico-chemical characteristics and microflora of *Lanhouin* samples collected from markets and processing sites. TBARS = Thiobarbituric acid reactive substances; TVN = total volatile nitrogen, TVC = total viable count; SA&CPS = *Staphylococcus aureus* and Coagulase Positive *Staphylococci*; CNS = Coagulase-Negative *Staphylococci*, LAB = lactic acid bacteria, MK = King fish *Lanhouin* from market, MC = Cassavafish *Lanhouin* from market; PC = Cassava fish *Lanhouin* from processing site, PK = king fish *Lanhouin* from processing site.

as cadaverine and putrescine. *B. cereus* was significantly and positively correlated with spermidine (r = 0.71, p<0.05) and positively correlated with histamine but not significantly (r = 0.44, p>0.05). But yeast and moulds was significantly and negatively correlated with putrescine (r = -0.76, p<0.05). These correlations indicate that B. cereus contributes to the formation of histamine and spermidine, but the presence of yeasts and moulds prevent the production of putrescine in Lanhouin. These observations are in agreement with Moreno-Arribas et al. (2000) and Kim et al. (2009) who reported a correlation between these biogenic amines indicated above and the presence of lactic acid bacteria during wine making, and the presence of *enterobacteria* during fish storage at a temperature greater than 4°C respectively. Similar observations were reported by various authors who indicated that some lactic acid bacteria and other microorganisms such as *Acinetobacter, Aeromonas, Bacillus, Clostridium, Escherichia* and *Pseudomonas* possessed amino acid decarboxylase activity and took part to the production of biogenic amines in fermented foods (Bover-Cid et al., 2001; Moreno-Arribas et al., 2000; Moreno-Arribas and Carmen Polo, 2008; Ntzimani et al., 2008; Kim et al., 2009).

Principal correspondence analysis on physico-chemical and microbiological characteristics of Lanhouin

The principal correspondence analysis (PCA) performed on the physico-chemical and microbiological characteristics of Lanhouin samples resulted in two axes accounting for 83.12% of the total variation, of which 45.95% was explained by the first axis (axis F1) and 37.17% by the second (axis F2) (Figure 1). Regarding the first axis, the cassava fish Lanhouin (MC) and the king fish Lanhouin collected from market (MK) were located in the right-hand part of the Figure 1, were the most dried samples, have the highest count of *Clostridium*,

Enterobacteria, S. aureus and CPS, and yeasts and moulds, and the highest content of citric acid, malic acid, formic acid and spermidine. This suggested that the market Lanhouin samples contained more spoilage microorganisms due to the fact that markets Lanhouin were more handled by the sellers and the customers at the selling places during purchasing.

Regarding the second axis, the cassava fish Lanhouin and the king fish Lanhouin collected from processing sites mainly located in the left hand part of the Figure 1, have the highest contents of histamine, cadaverine, putrescine; protein, TBARS, NaCl, acetic acid and the highest loads of CNS, lactic acid bacteria, *Bacillus cereus.* Among this group of micro-organisms, numbers are considered as biogenic amines producers (Kim et al., 2009; Suzzi and Gardini, 2003; Halasz et al., 1994). In addition, the highest contents of histamine in newly processed Lanhouin collected from processing sites could also be attributed to the fact that the amount of biogenic amines formed is influenced by factors such as the availability of free amino acids, which level may be high in newly processed Lanhouin than old and more dried Lanhouin collected from markets, although the water activity level of both Lanhouin samples appeared similar (Kerr et al. 2002).

Conclusion

The total viable counts of all Lanhouin samples are within acceptable limit. However, the presence of some bacteria such as *Clostridium perfringens* even though in few numbers in some samples showed that Lanhouin processing, handling and selling conditions need to be improved. The study also showed that protein, lipid and acidity index contents in Lanhouin samples were within acceptable limits but thiobarbituric acid (TBARS) contents were high as a reflection of some forms of spoilage. In addition, the pH values of the majority of samples were above 7. Acetic, citric and lactic acids are the predominant organic acids present in Lanhouin samples. For all Lanhouin samples the water activity (aw) values were slightly above 0.70. These levels of aw are relatively low to prevent enzymatic activity and microbial proliferation including food poisoning bacteria during storage. So, for the reengineering activities, one of the suitable manners to upgrade the quality of Lanhouin is to find a way to lower the pH of Lanhouin during processing. Low pH combined with low aw and appropriate packaging could allow the production of safe Lanhouin and improve as well the preservation of Lanhouin during storage.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This publication is an output from a research project funded by the European Union (FP7 245 – 025) called African Food Revisited by Research (AFTER - http://www.after-fp7.eu/).

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