

QUALITY CHARACTERISTICS OF *LANHOUIN* : A TRADITIONALLY PROCESSED FERMENTED FISH PRODUCT IN THE REPUBLIC OF BENIN

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ABSTRACT

Lanhouin, a fermented fish product is processed by spontaneous and largely uncontrolled fermentation. Disadvantages of this type of fermentation are that very little control can be exercised over the fermentation process and the product is often of variable quality with inherent risks of quality defects. Samples of *lanhouin* processed from cassava croaker /cassava fish (*Speudotolithus* sp.) or Spanish mackerel / king fish (*Scomberomorus tritor*), widely used as condiment in Benin, Togo, and Ghana, were purchased from processors and retailers in the processing sites and markets respectively, for product characterization. The samples were obtained from 20 retailers and 20 processors randomly selected in urban and rural processing sites and markets. The samples were transported under aseptic conditions to laboratory for analyses. The chemical composition of samples was determined using Pearson's and AOAC methods. The microbiological status was investigated according to the Nordic Committee on Food Analysis standard methods. The results showed that the pH values of the majority (78.5 %) of samples were above 7 and the water activity values ranged from 0.65 to 0.87. High level of free fatty acids (FFA) and thiobarbituric acid number (TBA) were also recorded for all the samples. Moisture, protein, salt, total volatile nitrogen and histamine contents ranged between 50.1-56.6 %, 24.6 - 26.5 %, 5.2 - 7.3 %, 294.5 - 374.5 mg N / 100 g and 21.4 -33.1 mg / 100 g respectively. Significant differences ($p < 0.05$) were observed on these parameters within and between species. The histamine contents in most of the samples (75%) exceeded the recommended level of 20 mg / 100 g. The total viable counts of the majority (83.5 %) of the samples were high. Micrococci and bacilli were the predominant organisms enumerated in the products. Their numbers ranged between 3.2×10^4 - 6.4×10^5 and 1.6×10^3 - 2.6×10^4 respectively. Few numbers of coliform bacteria and *Clostridium* spp. were enumerated in some of the samples (6 %). No *Salmonella* and no yeasts were found in any sample.

Key words: Fish, fermentation, *lanhouin*, quality

French

CARACTÉRISTIQUES DE LA QUALITÉ DE LANHOUIN: UN PRODUIT DE POISSON TRADITIONNELLEMENT TRAITÉ ET FERMENTÉ EN RÉPUBLIQUE DU BÉNIN

RÉSUMÉ

Lanhouin, un produit de poisson fermenté, est traité au moyen d'une fermentation spontanée et largement pas contrôlée. Les inconvénients de ce type de fermentation sont que très peu de contrôle peut être exercé sur le processus de fermentation et que le produit est souvent d'une qualité variable avec des risques de qualité inhérents. Des échantillons de *lanhouin* traités à partir du cassava croaker /cassava fish (*Speudotolithus* sp.) et du thazard tacheté / king fish (*Scomberomorus tritor*), largement utilisés comme condiment au Bénin, au Togo et au Ghana, ont été achetés auprès de traiteurs et détaillants sur des sites de traitement et sur des places de marché respectivement, pour la caractérisation des produits. Les échantillons ont été obtenus de 20 détaillants et de 20 traiteurs, sélectionnés au hasard sur des sites de traitement urbains et ruraux et sur des places de marchés. Les échantillons ont été transportés dans des conditions aseptiques au laboratoire pour une analyse. La composition chimique des échantillons a été déterminée en utilisant les méthodes de Pearson et de l'AOAC. L'état micro-biologique a été recherché selon les méthodes standard d'analyse du Comité Nordique sur l'Analyse des Aliments. Les résultats ont montré que les valeurs du pH de la majorité (78,5 %) des échantillons se situaient au-dessus de sept et que les valeurs des activités de l'eau variaient entre 0,65 et 0,87. Le niveau élevé des acides sans graisse (FFA) et du nombre d'acides thiobarbituriques (TBA) a été également enregistré pour tous les échantillons. La teneur en eau, en protéines, en sel, en nitrogène totale volatile et en histamine se situait entre 50,1 – 56,6 %, 24,6 – 26,5 %, 5,2 – 7,3 %, 294,5 – 374,5 mg N / 100g et 21,4 – 33,1 mg / 100g, respectivement. D'importantes différences ($p < 0,05$) ont été observées sur ces paramètres dans et entre les espèces. La teneur en histamine dans la plupart des échantillons (75 %) était supérieure au niveau recommandé qui est 20 mg / 100g. Les nombres totaux viables de la majorité (83,5 %) des échantillons étaient très élevés. Les *micrococci* et les *bacilli* ont été les organismes prédominants énumérés dans ces produits. Leurs nombres se situaient entre $3,2 \times 10^4$ - $6,4 \times 10^5$ et $1,6 \times 10^3$ - $2,6 \times 10^4$ respectivement. Très peu de nombres de bactéries coliformes et de *Clostridium* spp. ont été énumérés dans certains échantillons (6 %); cependant, aucun échantillon ne contenait de *Salmonella* et/ou de levure.

Mots-clés: Poisson, fermentation, *lanhouin*, qualité

INTRODUCTION

Fish and fish products play a significant role in the diets of the populations of West African countries. It is estimated that between 15 and 20 percent of all animal proteins come from aquatic sources [1]. However, post harvest losses of fresh fish are estimated to be about 20 % in West African countries [2]. While in developed countries the practice of cold storage limits the problem posed by the extreme perishability of fish, in tropical regions, particularly in West Africa, traditional processes such as drying, salting, smoking, fermentation and combinations of these treatments are used for fresh fish preservation [3]. A traditional fermented fish called *lanhouin* is mostly produced in the coastal regions of Benin. *Lanhouin* is widely used as condiment in urban and rural areas in Southern Benin and in the neighbouring countries of Togo and Ghana. *Lanhouin* is produced by natural and largely uncontrolled fermentation. In addition, the environment in which the fish is processed is generally unhygienic, paving the way for possible microbial contamination and evolution of food toxicants [4, 5, 6]. The result of this is quality defects, with occasional public health hazards.

For traditional *lanhouin* production, the fresh fish is scaled, gutted, washed and left overnight before the seemingly deteriorated fish is treated with salt and allowed to ferment for 3 to 8 days [7]. Since in the traditional processing of *lanhouin*, the fish must be in partially deteriorated form, chemical and microbiological changes are expected. Chemical compounds such as total volatile nitrogen (TVN) and biogenic amines (e.g histamine) which normally did not exist in the living tissues of fish are formed by autolysis and microbial actions [4, 5]. A particular problem in marine fish is the presence of trimethylamine oxide (TMAO) which is reduced by bacterial and enzymatic actions to trimethylamine (TMA) [8]. Gradual increases in total volatile nitrogen and thiobarbituric number contents and pH values were recorded during the processing of *momone*, a *lanhouin*-like fermented fish product [9,10]. Gradual decreases in moisture, fat and protein contents were also reported by various workers [9, 10]. Recent work on *momone* established that this fermented fish contained high levels of histamine (105–172 mg / 100 g) [10]. These levels exceed the hazardous limit of 200 mg / kg stipulated by the Australian Food Standards Code, the Food and Drug Administration (USA) and the European Economic Committee [11, 12, 13]. Thus, despite a lack of information on food poisoning caused by *lanhouin* in Bénin, there is a potential for sporadic amine poisoning. There is a need to investigate *lanhouin* to know the microflora present in this product and the biochemical changes which they bring about in the product. The present study on commercial *lanhouin* was carried out in order to determine the chemical and microbiological characteristics of the product.

MATERIALS AND METHODS

Materials

Twenty five samples of *lanhouin* obtained from cassava fish (*Pseudotolithus* sp.) and twenty five others from king fish (*Scomberomorus tritor*) were purchased from processors and retailers in the markets and the processing sites. The samples were collected into sterile plastic bags and stored in icebox filled with ice and transported to the laboratory for analyses.

Methods

Microbiological analyses

Ten (10) grams of each sample (steaks cut from the head, middle and tail region of the fish) were weighed into a sterile stomacher bag with 90 ml of sterile diluents containing 0.1% peptone, 0.8 % NaCl with pH adjusted to 7.2. The mixture was then macerated for 2 minutes in a stomacher (Lab Blender, Model 400). 1 ml of the homogenate was serially diluted in aseptic conditions and used for enumeration of microorganisms. Total aerobic mesophilic and halophilic bacteria, coliforms and faecal coli, *Clostridium* spp. and *Staphylococcus aureus* were enumerated according to the Nordic Committee on Food Analysis official methods [14, 15, 16, 17] using Plate Count Agar (Oxoid CM 463), Plate Count Agar supplemented with 15 % salt, Violet Red bile Agar (Oxoid CM107), Iron Sulphite Agar (Oxoid CM 79) and Baird Parker (Oxoid CM 275) respectively. PCA plates were incubated at 30°C for 3 days; plates for coliform and faecal coli were incubated for 24 h at 30°C and 44°C respectively. Baird Parker plates and *Clostridium* plates were incubated at 37°C for 24 to 48 h and 48 h respectively.

Salmonella were investigated on Xylose-Lysine-Desoxycholate Agar (Oxoid CM 469) after pre-enrichment in Buffered peptone (Oxoid CM 509) and selective enrichment in Rappaport-Vassiliadis Broth (Oxoid CM 669) [18]. Micrococci were enumerated according to the method described by Rodriguez *et al.*, (1994). 0.1 ml portion of appropriate dilutions were plated on Mannitol Salt Agar (MSA, Oxoid CM 85) which were then incubated for 48 h at 30°C [19]. Bacilli were investigated on Dextrose Tryptone Agar (DTA, Oxoid CM 75) and the plates were incubated for 48 h at 35°C [20]. Yeasts and mould were enumerated on Malt Extract Agar (MSA, Oxoid CM 59) containing 2 ml of sterilised lactic acid 10 % per 100 ml of medium; the plates were incubated at 25°C for 3-5 days [21]. The colonies appearing were counted as colony forming units (cfu) per gram wet weight of sample. All tests were carried out in duplicate.

Physico-chemical analyses

pH of samples was measured with a pH-meter (Hanna Instrument HI 9318) on a mixture of 20g of blended fish meat and 80 ml of distilled water. Water activity (A_w) was measured with a thermo-hygrometer recorder C056696 according to the Troller method [22]. The sample was sealed in a container, which is held at a constant temperature until the water vapour in the air reaches equilibrium with the moisture in

the product. A probe determines the equilibrium relative humidity (ERH) above the product and the water activity was determined from the relationship $ERH/100$.

Free fatty acid (FFA), total volatile nitrogen (TVN) and thiobarbituric acid number (TBA) were determined according to Pearson methods [23]. For FFA, 1g of crude fat extract from the fish samples using the Soxhlet method (AOAC, 1995) was mixed with a neutral solvent (alcohol + diethyl ether; 50:50) and titrated with aqueous 0.1M NaOH.

For TVN, 10g of sample was blended and added to 300 ml tap water and 2 g magnesium oxide in a distilling flask of macro-Kjeldahl for distillation. The distillate collected in 2 % boric acid was titrated with 0.1N H_2SO_4 . The TVN as mg N/100g was obtained multiplying the titre (less blank) by 14.

The TBA was determined on 10g of sample macerated with deionised water. The mixture was transferred into a distilling flask for distillation. 5 ml TBA reagent was added to 5ml aliquots of distillate and after heating and cooling the absorbance of the solution was measured at 538 nm using a spectrophotometer Shimadzu UV-240.

Moisture, protein, salt and histamine were determined using AOAC methods: 950.46, 981.10, 937.09 and 977.13 respectively [24].

Statistical analyses

Statistical package for Social Science (SPSS version 10) was used. Data analyses involved one-way analysis of variance (ANOVA). Means difference were determined using Duncan's Multiple Range Test. Significant difference was established.

RESULTS

Physico-chemical characteristics of samples

The chemical composition of *lanhouin* samples collected is showed in Table 1.

Moisture content

The moisture contents of samples varied between 45.3 and 61.6 % with a mean of 50.1 and 56.6 % for samples obtained from cassava fish and king fish, respectively. Significant difference ($p < 0.05$) was observed within and between species. The highest moisture contents were recorded on *lanhouin* samples prepared from king fish (Table 1).

Water activity

The highest values of water activity (A_w) were recorded on the samples obtained from king fish. The water activity values ranged from 0.65 to 0.77 for cassava fish and 0.67 to 0.87 for king fish (Table 1). Significant difference ($p < 0.05$) was observed between the measurements.

pH

pH values of the majority (78.5 %) of samples were above 7 and varied between 6.7 and 7.9 for cassava fish, and 7.3 and 7.9 for king fish (Table 1). There was a statistically significant difference ($p < 0.05$) between these values.

Free fatty acids and thiobarbituric acid numbers

The free fatty acids (FFA) contents of *lanhouin* samples obtained from cassava fish varied between 11 and 14 % oleic acid whereas that obtained from king fish varied between 27.2 and 36.6 %. The thiobarbituric acid values (TBA) ranged from 5.3 to 6.1 and 7.2 to 9.4 mg malonaldehyde /kg for cassava fish and king fish samples respectively (Table 1). Highest values of both FFA and TBA were recorded on samples prepared from king fish. Significant difference ($p < 0.05$) was observed among the data within and between species

Total volatile nitrogen

The total volatile nitrogen (TVN) contents of samples ranged from 264.7 to 389.8 mg N/ 100 g from one species to another with the average of 294.5 and 374.5 mg N/ 100 g for cassava fish and king fish respectively (table1). The highest values of TVN were recorded on the samples obtained from king fish.

Protein content

The protein contents in *lanhouin* samples prepared with cassava fish varied between 23.4 and 29.6 % while that of king fish ranged from 20.9 to 28.3 % (table 1). No significant difference was noted ($p > 0.05$) between species but a significant difference ($p < 0.05$) was observed within species.

Sodium chloride content

The salt concentrations determined in the samples ranged between 5.2 ± 1.0 % and 7.3 ± 1.6 % for cassava fish and king fish respectively (Table 1). The differences in these values were statistically significant ($p < 0.05$).

Histamine content

The histamine contents of *lanhouin* samples prepared with cassava fish ranged between 17.4 and 25.4 mg / 100g whereas that obtained from king fish varied from 26.5 to 39.7 mg/ 100g (Table 1). 75 % of samples obtained from cassava fish showed histamine contents higher than the maximum allowable level of 20 mg / 100g while all the samples (100 %) from king fish showed higher content than the recommended level. The highest levels of histamine were recorded on the samples obtained from king fish.

Microbiological analyses

The microbiological status of samples is summarized in Table 2. The total viable count of the majority (83.5 %) of samples was high. Count (Log cfu/g) of total aerobic mesophilic ranged from 5.4 to 6.6 and 6.2 to 7.8 for samples of cassava fish and king fish respectively. Halophilic bacteria count (Log cfu/g) varied between 4.5 and 5.5, and

5.4 and 6.3, respectively, from one species to another. Micrococci and bacilli ranged from 5.0 ± 0.6 and 5.4 ± 0.4 , and 3.7 ± 0.5 and 4.1 ± 0.3 , respectively (table 2). Coliforms and faecal coliform counts (Log cfu/g) were lower than 1 for most of the samples; however in 6 % of the samples the coliform loads (Log cfu/g) ranged between 1.47 and 1.84. Mould counts (Log cfu/g) were lower than 1 for all samples while *Clostridium* spp. counts varied between 1.68 and 1.8. *Staphylococcus aureus* was absent in the majority (82.3 %) of the samples; however *Staphylococcus* counts (Log cfu/g) lower than 1 were observed in the remaining of the samples. No *Salmonella* and no yeasts were detected in all the samples.

DISCUSSION

Chemical analysis

The variation in moisture contents of samples could be the result of variable drying time and level and type of salt used for the curing. However, the moisture content seems to be an inexact indicator of the susceptibility of a product to undergo microbial spoilage. A major factor, which determines the microbial, chemical and enzymatic stability of foods, is the water activity (A_w) [22].

The water activity values of the majority of *lanhouin* samples were around 0.70 or slightly above 0.70. These values of water activity are relatively low to support enzymatic activity and microbial proliferation including food poisoning bacteria during storage. The major histamine producing bacteria are active within A_w range of 0.91 to 1.0 [22].

The pH values of the majority of samples were above 7. Similar higher values of pH were reported on *momone*, a *lanhouin*-like fermented fish of Ghana [9, 10, 25]. No literature on the recommended pH range of *lanhouin* is available. Similar fermented fish known as *Pedah siam* is processed in Thailand. For this product fresh fish is fermented unlike the partially deteriorated fish used for *lanhouin* processing. The standard pH requirement for *Pedah siam* is 6.0 - 6.4 with a pH of 6.5 or higher considered as indicative of poor quality [26]. But, since in the processing of *lanhouin*, a seemingly deteriorated fish must be used, the high pH values around 7 or above 7 obtained is expected and may be considered as the usual pH value for this fermented fish.

Free fatty acid contents of samples were very high for both species of fish. High level of free fatty acids is an indication of microbial spoilage activity [8, 23, 27]. Most fat acidity begins to be noticeable to the palate when the free fatty acid values calculated as oleic acid is about 0.5 - 1.5 % [23]. Thiobarbituric acid numbers (TBA) of all samples showed high level (5.7 - 8.3) as a reflection of chemical spoilage activity with its attendant rancidity.

As expected, the total volatile nitrogen (TVN) contents were high for all samples. These values of TVN could be compared favourably as similar to values obtained by

other workers [10, 25]. Level of total volatile nitrogen (TVN) in fish is commonly used as a spoilage indicator [6, 23, 28]. TVN measurements indicate the extent of the breakdown of proteins due to bacterial and enzymatic action, leading to amines production and thus a low nutritional value of the product [23, 26, 28]. Pearson (1976) suggests that for white-fleshed fish, TVN levels below 200 mg N/kg indicate that the fish is fresh, whereas the fish would be rejected for human consumption when the TVN level exceeds approximately 500 mg N/kg [6].

Protein values obtained for different samples agree with those reported by other workers [10, 25]. However higher protein content of 57% was also obtained mainly on fish fermented for two days [9]. These variations observed in protein levels could be probably due to the variation in fish species, the level of salt used and the period of fermentation, which determined the degree of proteolytic activity during the processing. Salt contents of all *lanhouin* samples appeared low when compared to the values of 10 and 15% found in *momone* [25]. However salt concentration of 4- 6% were also reported in *momone* [10]. The salt concentrations of 5.2-7.3% recorded on *lanhouin* samples are too low to inhibit microbial histidine decarboxylase activity since histamine production by bacteria may occur at 8 % salt and up to 12 % salt [4, 29].

The histamine levels in the samples exceeded the Australian Food Standards Code, the Food and Drug Administration (USA) and the European Economic Community (EEC) safe level of 200-mg/kg fish muscle [11, 12, 13]. The high level of histamine in the samples could be an indication of mishandling during storage and processing and could also be the result of the low salt concentration of samples allowed microbial activity to occur leading to the formation of histamine [4, 6, 30].

Microbiological analysis

Counts of total aerobic mesophiles on samples showed a high microbial population. The predominant microorganisms were micrococci followed by bacilli. Various authors have reported similar microflora in *momone* [9, 10, 25] and in other fermented fish products [5]. The low counts of coliforms and faecal coli in most of the samples showed that *lanhouin* is weakly subjected to faecal contamination. The presence of coliform bacteria, *Staphylococcus aureus* and *Clostridium* spp. even though in few numbers in some of the samples is still significant and show that there is need for improved handling and processing procedures of *lanhouin*. The absence of the pathogen *Samonella* in all the samples is worth noting as this may have been the result of the salt concentration (5.2-7.3%) of the samples.

CONCLUSION

This study showed that free fatty acids, thiobarbituric acid numbers and total volatile nitrogen contents of samples were very high as a reflection of some forms of spoilage. Salt contents of the *lanhouin* samples were too low to inhibit microbial histidine decarboxylase activity. In addition, histamine levels in most of the samples were higher than maximum allowable level of 20 mg / 100g fish. In general, samples analysed were

found not to be safe microbiologically and this could be a consequence of unhygienic conditions observed during processing and marketing. However, pathogens such as *Salmonella* were not found but *Clostridium* species were present in some of the samples. Bacilli and micrococci generally considered as a normal microflora of such products were present in all the samples. It is expected that controlled material handling and fermentation will lead to lower levels of histamine and better microbial status in the product.

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Table1

Chemical characteristics of *lanhouin* on sale

	Cassava fish (lean) n = 25	King fish (fatty) n = 25
Moisture (%)	50.1 ± 4.9a	56.6 ± 5.0b
Water activity	0.71 ± 0.06a	0.77 ± 0.10 b
pH	7.3 ± 0.6 ¹ a	7.6 ± 0.3b
Free fatty acids (% oleic acid) ²	12.5 ± 1.5a	31.9 ± 4.7b
Thiobarbituric acid value (mg malonaldehyde / kg) ²	5.7 ± 0.4a	8.3 ± 1.1b
Total volatile nitrogen (mg N/100g) ²	294.5 ± 29.8a	374.5 ± 15.3b
Crude protein (% nitrogen × 6.25)- (g/100 g) ²	26.5 ± 3.1a	24.6 ± 3.7a
Salt (%) ²	7.3 ± 1.6a	5.2 ± 1.0b
Histamine (mg/100g) ²	21.4 ± 4.0a	33.1 ± 6.6b

n: number of samples analysed, each sample in duplicate;

¹Means ± Standard Deviations (SD); ²Wet weight basis

a, b: Means with different letters in a row are significantly different (p < 0.05)

Table 2
 Microbiological status of *lanhouin* on sale (Log cfu/g)

	Cassava fish n = 20	Kingfish n = 20
Total aerobic mesophilic count	6.0 ± 0.6 ¹ a	7.0 ± 0.8b
Halophilic count	5.0 ± 0.5a	5.8 ± 0.4a
Micrococci	5.0 ± 0.6a	5.4 ± 0.4a
Bacilli	3.7 ± 0.5a	4.1 ± 0.3a
Coliforms	< 1	< 1
Faecal coli	< 1	< 1
Moulds	< 1	< 1
<i>Clostridium</i> spp.	1.6 ± 0.2a	1.8 ± 0.2a
<i>Salmonella</i> ^c	absent	absent
<i>Staphylococcus aureus</i>	absent	absent

n: number of samples analysed, each sample in duplicate

¹ Means ± Standard Deviations (SD); ^c Search in 25 g sample

a, b: Means with different letters in a row are significantly different (p < 0.05)

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