Research Article

Physico-chemical and Microbiological Characterisation of Pheasant and Salted Fish (*Lanhouin*) Produced and Sold in Lomé

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Submission Date: 19-08-2022; Revision Date: 02-09-2022; Accepted Date: 20-10-2022.

ABSTRACT

Aim: To assess the quality of *Lanhouin* produced and sold in Lomé, physico-chemical and microbiological characterisations were carried out on this commodity. **Materials and Methods:** 45 samples of pheasant and salted fish obtained from the species *Pseudotolithus senegalensis*, *Balistes capriscus*, *Scomberomus tritor*, *Decacttylus galéides*, *Cheilopognon nigricans* were collected at Katanga and at the Kodomé market. The standardised routine methods described by the Association Française de Normalisation were used to search for the suspected germs. Physico-chemical analyses were carried out on 10 of the 45 samples taken and concerned water content, fat and protein levels obtained by the AOAC methods. **Results:** The results showed that samples have water contents ranging from 51% to 65.85% and fat contents from 1.24% to 2.53%. Protein content varied from 33.41% to 53.69%. Microbiological analyses revealed the absence of salmonella. Hygienic quality of the samples analysed was unsatisfactory at 24.44% in relation to total mesophilic flora, at 88.89% in relation to total coliforms, at 33.33% in relation to *Escherichia coli,* at 62.22% and 8.89% respectively in relation to Sulphite Reducing Anaerobes and *Staphylococcus aureus*. Yeasts and moulds were found in all the samples collected, which indicates a potential risk of altering the hygienic and marketable qualities of the products. The presence of lactic acid bacteria found in the *Lanhouin* could result in the possible presence of biogenic amines. **Conclusion:** These results imply the need to rigorously apply good manufacturing and hygienic practices in order to improve the sanitary quality of pheasant and salted fish for the well-being of its consumers.

Keywords: *Lanhouin*, Lomé, Microbiology, Physico-chemical, Quality.

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INTRODUCTION

In Togo, *Lanhouin* is salted, pheasant fish. Sold in traditional markets, it is used as a flavour enhancer by the populations of southern Togo. It is used in most preparations of leafy vegetable sauce, seed sauce, soup and other dishes. Nutritionally, *Lanhouin is* a source of protein and polyunsaturated fatty acids.[1] Moreover, its manufacturing process allows this food stuff to be preserved for a few days to a few months using rudimentary equipment. This artisanal technique, which costs little in terms of investment, provides significant income for women, who are the main producers and sellers.[2-4]

In Benin, the term *Lanhouin* refers to fish that is fermented, salted and dried.[5] The technique practiced in Lomé, Togo, is slightly different as drying is absent.[4] In addition, studies conducted in Benin, Senegal and Côte d'Ivoire have shown highly variable results on

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DOI: 10.5530/ajbls.2022.11.89

the microbiological and physicochemical aspects of fermented fish. This variability in manufacturing methods observed by Fall *et al*.[6] led them to conclude that studies should be conducted for each product in each country, taking into account the type of fish, climatic conditions, desired taste and processing technique. Furthermore, the environment surrounding the production of *Lanhouin* is completely unhealthy and the key stages of its manufacture, namely maturation, salting and fermentation, are spontaneous and therefore completely uncontrolled.[2,4] Germs indicative of poor hygiene and high levels of biogenic amines are found in *Lanhouin* produced in Benin. In Togo, only one study on the hygienic quality of this fermented and salted fish was conducted by Abdelsalam *et al*.[7] despite its economic importance and the microbiological and toxicological risks that this foodstuff could carry. The present study was therefore conducted to characterize the physico-chemical and microbiological properties of *Lanhouin* produced and sold in Lomé.

MATERIALS AND METHODS

Biological materials

The study focused on samples of *Lanhouin* produced from five (05) fish species. These species were selected because they are highly appreciated by consumers. Their availability at the time of the study also influenced their choice. The five fish species were as follows: *Cheilopogon nigricans* (flying fish), *Pseudotolithus senegalensis* (sea bass), *Balistes capriscus* (triggerfish), *Scomberomorus tritor* (mackerel), *Decadacttylus galeides* (false captain).

Setting up the sample size for physico-chemical and microbiological analyses

A total of forty-five (45) samples of *Lanhouin* were taken from the fishermen's village of Katanga and the market of Kodomé. Katanga is located in the port area, a short distance from the old fishing port in Lomé. The Kodomé market, however, is located in the Tokoin-Ouest area of Lomé. These two locations were chosen because of the large number of "*lanhouin*" producers and traders found there. These samples were taken at random within each species according to the availability of fish. Their distribution is presented in Table 1.

Determination of physico-chemical contents

The physico-chemical analyses mainly concerned the determination of water, fat and total nitrogen contents.

Determination of water content

The water content was determined according to the method of the Association of Official Analytical Chemists (AOAC). The samples were oven dried at 105°C to constant mass. The content is determined by the following formula:

$$\text{Water}\,(\%) = \frac{M_1 - M_2}{M_1} \times 100$$

Where M_1 is the mass of the sample before steaming M_2 the mass of the oven-dried *Lanhouin*

Table 1: Distribution of *Lanhouin* samples according to the fish species used.

Species used as raw material for *lanhouin*	Common name	Units withdrawn
Pseudotolithus senegalensis	Bar	10
Decadacttylus galeides	False Captain	10
Scomberomorus tritor	Mackerel	10
Cheilopogon nigricans	Flying fish	10
Balistes capriscus	Ballista	05

Physico-chemical analyses were carried out on ten (10) of the 45 samples taken, i.e. two samples per species.

Determination of fat content

The fat content is determined using the Soxhlet method.[8] The oil from the salted, pheasant fish is dissolved in hexane, filtered and recovered after evaporation of the solvent. The solvent-free fat is weighed and the extraction yield is determined.

Determination of protein content

The Kjeldahl method[8] was used for the determination of crude protein content from the dry matter of *Lanhouin*.

Microbiological analysis

Samples of *Lanhouin were* taken in clean bags, labelled and placed in the cooler before being transported to the LAMICODA-ESTBA/UL laboratory. The microbiological analyses of the pheasant and salted fish were carried out according to the standardised routine methods described by the Association Française de Normalisation (AFNOR) and adopted by West African Economic and Monetary Union (WAEMU). Thus, 25 g of salted pheasant fish were taken and added to 225 ml of buffered peptone water. From the stock solution of 10^{-1} obtained after homogenisation, subsequent dilutions were made up to 10^{-5}. The germs sought for microbiological quality are those retained by the European Union (EU) criteria for fermented fish products. These are total germs (NF V08-051), total coliforms (NF V08-050), *E. coli* (NF EN ISO 6579-1), S. *aureus* (NF V 08-057), *Salmonella sp* (NF V 08-052) and sulphite-reducing anaerobes (XP V08-061).

The analytical variability, associated with the three-class design, was considered for the interpretation of the results.

Other germs such as lactic acid bacteria (30°C) and yeasts and moulds (30°C XP-V08-059) were also tested in order to determine the flora affecting the marketability of *Lanhouin*.

RESULTS

Physico-chemical characteristics of the *Lanhouin* of Lomé

The results of the physico-chemical tests are shown in Table 2. The water content of the samples varies between 51% and 65.85% in relation to the fish species used.

Microbiological characteristics of Lomé Lanhouin

The number of germs per fish species used for the production of *Lanhouin* is presented in Table 3. It varies from sample to sample. No species of fish seems to be more specifically contaminated than another by aerobic mesophilic flora, total coliforms, *E. coli*, anaerobic sulphite-reducers. *Staphylococcus aureus* are found in the *Lanhouin* samples obtained from *Pseudotolithus senegalensis, Decadacttylus galeides* and *Scomberomorus tritor*. *Salmonella* were absent from all samples. The assessment of the hygienic quality induced by the presence of the germs enumerated in the different samples is shown in Table 4. The rate of unsatisfactory samples in relation to the total mesophilic flora varied from 0% with the species *Scomberomorus tritor* to 50% with the species *Cheilopogon nigricans*. The presence of these germs resulted in an acceptable hygienic quality of 46.67% for all samples.. For total coliforms, the unsatisfactory hygienic quality varied from 80% for samples of *Scomberomorus tritor* and *Balistes caprisus* species to 100% for those of *Cheilopogon nigricans* species. Sulphite reducing anaerobes induced

Table 2: Physico-chemical analyses of *Lanhouin* produced and sold in Lomé.

Species of fish used (Common name)	Water content (Mean ± SD)	Fat content (Mean ± SD)	Crude protein content (Mean ± SD)
Decadactylus galeides (False Captain)	51 ± 4.11	02.22 ± 1.73	41.99 ± 0.46
Scomberomorus tritor (Bar)	55.66 ± 0.24	02.23 ± 0.19	37.71 ± 0.15
Pseudotolithus enegalensis (Mackerel)	57.27 ± 0.54	02.53 ± 0.86	49.46 ± 1.27
Cheilopogon nigricans (Flying fish)	61.85 ± 6.08	01.24 ± 0.25	53.69 ± 2.08
Balistes capriscus (triggerfish)	65.85 ± 8.54	01.24 ± 0.25	33.41 ± 0.14

The fat contents in the different samples vary from 1.24% to 2.53%. Protein analysis shows a variation from 33.41% to 53.69%.

Table 3: Number of germs found in *Lanhouin* samples by fish species.

Searched for germs	Number of germs (CFU/g)															Criteria
	Pseudotolithus senegalensis			*Cheilopogon nigricans*			*Decadacttylus galeides*			*Scomberomorus tritor*			*Balistes capriscus*			
	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	
FAMT at 30°C (x10^6)	0,8	400	41,56	4,6	69,6	18,68	0,2	16,4	5,91	0,6	9,1	3,98	1,6	49,4	15,6	1
CT (30°C)	30	41000	7023	108	73000	21350,8	0	78000	14050	00	26000	4030	00	100000	34600	<10
E. coli (44°C)	0	300	103,1	0	200	43	0	700	188	00	210	53	0	100	20	<10
ASR (44°C)	0	40000	5662	0	11500	2067	0	200	50	00	6600	1040	0	10000	4740	<10
S. aureus (37°C)	0	1000	100	0	0	0	0	1000	100	00	3000	400	00	00	00	<100
Sal (37°C)	0	0	0	0	0	0	0	0	0	00	00	00	00	00	00	Absent in 25g

FAMT: Total mesophilic aerobic flora; **TC:** Total coliforms; ***E. coli*:** *Escherichia coli*; **SRP:** Sulphite-reducing anaerobes; ***S. aureus*:** *Staphylococcus aureus*; **Sal:** *Salmonella*; **S:** Satisfactory; **A:** Acceptable; **NS:** Unsatisfactory; **Min:** Minimum; **Max:** Maximum.

Table 4: Assessment of the hygienic quality of *Lanhouin* samples in relation to the different fish species.

Searched for germs	Quality of Samples from *"Lanhouin*														
	Pseudotolithus senegalensis			*Decadacttylus galeides*			*Scomberomorus tritor*			*Cheilopogon nigricans*			*Balistes capriscus*		
	S	A	NS	S	A	NS	S	A	NS	S	A	NS	S	A	NS
FAMT at 30°C	40	30	30	40	50	10	40	60	00	00	50	50	20	40	40
TC (30°C)	00	10	90	10	00	90	20	00	80	00	00	100	20	00	80
E. coli (44°C)	40	20	40	50	10	40	70	00	30	60	10	30	80	00	20
ASR (44°C)	30	00	70	70	00	30	40	00	60	20	00	80	20	00	80
S. aureus (37°C)	90	00	10	90	00	10	80	00	20	100	00	00	100	00	00
Sal (37°C)	100	00	00	100	00	00	100	00	00	100	00	00	100	00	00

FAMT: total mesophilic aerobic flora; **TC:** total coliforms; ***E. coli:*** *Escherichia coli*; **SRP:** Sulphite reducing anaerobes; ***S. aureus:*** *Staphylococcus aureus*; ***Sal:*** *Salmonella*; **S:** satisfactory; **A:** acceptable; **NS:** unsatisfactory.

Table 5*:* Number of other germs found in *Lanhouin* samples.

Fish type	Yeasts and moulds	Lactic acid bacteria
Mackerel	3826.10^2	45.10^2
Flying fish	1616.10^2	244.10^2
False Captain	1115.10^2	$130\ 10^2$
Bar	$370\ 10^2$	356.10^2
Ballista	$810\ 10^2$	130.10^2

an unsatisfactory hygienic quality ranging from 30% to 80%.

The other germs investigated were yeasts and moulds and lactic acid bacteria. The counts are shown in Table 5. Fungal flora (yeasts and moulds) were detected in all samples. The average ranged from $3.70.10^4$ to $3.83.10^5$ CFU/g (Table 5).

DISCUSSION

Physico-chemical analysis of *Lanhouin*

The water content in this study is high compared to samples from Benin[2] and Senegal.[9] The drying practised in these two countries in the production of this condiment in Benin and Senegal could justify this difference. Another reason could be the uncontrolled salting during the production process. The highest water content recorded with the *Balistes capriscus* species (65.85%) would probably be due to its harder skin which would not allow good permeability of the salt into the fish flesh. With such high water content, preservation at ambient temperatures in Togo cannot be guaranteed without risks to the microbiological quality of the *Lanhouin*. The fat contents seem low compared to the lipid contents of these fish. Indeed, the *Lanhouin* obtained from mackerel, which is supposed to be a fatty fish (12% lipids)[1] has a fat content of 2.53%. It is therefore possible that the lipids have been modified.

Protein analysis results indicate a higher content than that of Benin's *Lanhouin* which is 28.7%.[2] However, these contents are evaluated in relation to the dry matter. Moreover, the elimination of water bound to the protein molecules after the addition and penetration of salt reduces the total weight, causing the proteins in the pheasant and salted fish to clump together.[10]

Hygienic quality of Lomé *Lanhouin*

Anihouvi *et al*.[2] found that the total aerobic mesophilic flora (FAMT) is at an acceptable level for 40% of the samples. The high presence of total coliforms and sulphite reducing anaerobes in ours samples can be justified by the lack of good hygienic and manufacturing practices on the part of the processors.[4] On the other hand, Abdelsalam *et al*.[7] found 100% and 80% satisfactory hygienic quality in fermented, salted and dried fish respectively. This difference could be due to the sample size of the present study which is three times larger than that of the study by Abdelsalam *et al*.[7] The drying practiced on the samples of the previous study too could explain this difference. However, drying alone is not sufficient since the same authors found in fermented and salted but not dried fish, satisfactory compliance rates of 93.33% for total coliforms and 73.33 for sulphite reducing anaerobes. As the latter are ubiquitous germs and indicators of technological failure, their presence in the *Lanhouin* samples would probably be related to handling on the floor, as well as the presence of flies on the production sites, not to mention the recycled and poorly stored salt used for production. Hygienic conditions are the basis for obtaining safe food products. They must be applied throughout their processing.[5]

These variations in the number of germs from sample to sample and in the rate of unsatisfactory samples appear to be more related to the handling of the samples than to the raw material used to produce the

pheasant fish. The rate of unsatisfactory samples with respect to *E. coli,* varies from 20 to 40% depending on the species of *Balistes caprisus* on the one hand and *Pseudotolithus senegalensis* and *Decadacttylus galeides* on the other hand (Table 4). *E. coli* is a bacterium often encountered in the gastrointestinal tract of humans and warm-blooded animals, so its presence in some samples is a prime indicator of faecal contamination. These results contrast with those that did not find *E. coli in* fermented and salted fish samples in Lomé.[7] *Staphylococcus aureus* is absent in the species *Cheilopogon nigricans* and *Balistes capriscus*. For the three other species, the rates are unsatisfactory, between 10 and 20% (Table 4). Of the 45 samples analysed, 41 or 91% were of satisfactory hygienic quality with respect to *Staphylococcus aureus*. Abdelsalam *et al*.[7] had determined a satisfactory compliance rate of 100% on *Lanhouin* despite the difference in sample size. However, the presence of *Staphylococcus* in some samples suggests that hygiene measures should be taken as these germs in addition to being pathogenic can produce biogenic amines such as histamine from free amino acids.[11,12]

Salmonella was absent from all samples analysed. Similar results were found in Lomé,[7] in Côte d'Ivoire,[13] in Benin,[14] and in Senegal.[3] All these results guarantee the non-existence of a risk linked to the presence of these pathogens in pheasant and salt fish.

As production is generally done in the air, fish are subject to multiple sources of contamination, including yeast and mould. In addition, the old loincloths and jute bags used to cover the fish can be a source of mycological contamination. The presence of yeasts and moulds could indicate the production of dangerous catabolites such as histamine from the decarboxylation of histidine.[15]

The presence of lactic acid bacteria in most samples is estimated at between $4.5.10^3$ and $3.56.10^4$ CFU/g. The lactic acid bacteria are capable of synthesising biogenic amines.[15,16] Indeed, they produce the histidine decarboxylase responsible for the formation of histamine. Histamine poisoning is considered to be the most common food poisoning resulting from fish consumption involving biogenic amines.[17] Furthermore, the maturation and fermentation of fish are favourable conditions for proteolysis and decarboxylation of amino acids.[16,18,19]

CONCLUSION

Lanhouin is a condiment that is highly prized in most countries of the Gulf of Guinea. The physico-chemical analysis of some samples, taken in Lomé, Togo, revealed a low-fat content, a relatively high protein content and an equally high-water content compared to the results of the sub-region.

The germs that indicate a lack of hygiene are present in all the samples analysed. Similarly, yeasts and moulds were found in all the samples of *Lanhouin* analysed. All these factors, apart from the toxicity of some, are favourable to the production of biogenic amines and therefore likely to make this condiment dangerous for consumers.

Standardisation of the production method and hygienic improvement of the production conditions are therefore necessary to guarantee a healthy *Lanhouin* for consumption.

ACKNOWLEGEMENT

The authors would like to thank the producers and sellers of *Lanhouin* in Lomé who participated in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Authors' Contributions

Conceived and designed the experiments: A.M-L and D.B.R. Sampling and questionnaire administration: A.M-L. Performed the experiments and analyzed the data: A.M-L, D.B.R., S K., A.K. and K-D M. Contributed reagents/materials/analysis tools: K.D.S, A.Y and G.M. Wrote the paper: D.B.R, A.M-L. M.M., S. K. and A.T.

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Cite this article: Dossou RB, Atakora M, Melila M, Melila M, Soncy K, Kangni-Dossou M, Karou DS, Ameyapoh Y, Gbeassor M. Physico-chemical and Microbiological Characterisation of Pheasant and Salted Fish (*Lanhouin*) Produced and Sold in Lomé. Asian J Biol Life Sci. 2022;11(3):678-83.