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ASSESSMENT OF THE HYGIENIC QUALITY OF FERMENTED AND DRIED SALTED FISH SOLD IN LOME, TOGO

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Abstract

Because of its protein richness and all its nutritional value, fish is widely applied either directly or indirectly, or after processing in the human diet. Thus, the need for this product at all times pushed the man to develop conservation methods, including salting, drying and fermentation. The effectiveness of these methods often hides the defective hygienic quality of products derived from them. This study of fifteen (15) samples of dried salted fish and fifteen (15) samples of fermented fish 'lanhouin' collected in two (2) markets of Wholesale in Lomé, aims to assess the hygienic quality of these two (2) product types. Indicators sprouts breach hygiene rules and pathogens were searched using the standard routine of the French Standards Association (AFNOR) methods. An estimate of the profile of the microbial product was made by Gram staining and catalase tests and oxidase.

The results show that the samples of dried salted fish and fermented fish analyzed are not contaminated with Salmonella, thermotolerant coliforms, *Escherichia coli*, yeast and mold. Regarding dried salted fish, the rate of non-compliance identified by the criteria is 26.66 % for the total bacteria (30 °C) and 20% for sulphite-reducing anaerobes, and it is about 20% for *S. aureus*. All products comply vis-à-vis the total coliforms. On fermented fish, the rate of non-compliance is 26.66% and 6.66 % respectively compared to total bacteria (30 °C) and total coliforms (30 ° C) and 26.66% compared with sulphite-reducing anaerobes . None of these samples containing *S. aureus*. Furthermore, Gram positive bacilli are predominant to 84.61% against 15.38% Gram positive cocci, in dried salted fish (for 26 germs isolated); and 88.46%, against 11.53% of Gram-positive cocci in fermented fish (for 26 germs isolated). The majority of Gram-positive bacilli are positive catalase and negative oxidases. Cocci are catalase and oxidase positive. So it would be interesting to be a characterization of biochemical and molecular plan to learn more about the species and strains of the bacilli and cocci Gram positive colonizing these two types of fish.

Key words: fish, salting - drying, fermentation, hygienic quality, bacteria

1. Introduction

Global fish consumption is increasing due to its nutritional quality (Medale et *al*., 2008). Poorly preserved, fish rapidly degraded by the action of germs intrinsic as well as extrinsic origin germs affecting its quality and causing poisoning to consumers (Rozier, 1996). Artisanal fish processing, extends the shelf life of fish products (Oumoulkhairy and Yvette, 2009). These techniques based on smoking, salting, drying and fermentation (Momar, 2007; Michel, 2005; Boyd et *al*., 1992; Gerard, 1989; Fellows, 1997). This transformation gives very interesting products that are the staple food in many countries. In the southern regions of Ecuador, northern Peru and valleys of the eastern Andes in Bolivia, dried salted fish is consumed more heavily during Lent Easter (UNICEF, 1989). In the sub-region of West Africa, the products obtained by fermentation, including "Gaj" in Senegal, "Adjuevan" in Cote d'Ivoire, "Momone" in Ghana, the "Stink fish" in Liberia, and "Lafi" in Guinea (Laurence et *al*., 2005; Koffi, 2011), are highly prized commodities for consumers. In Benin and Togo the "lanhouin" salted fish, fermented and dried, is used as a flavoring (Anihouvi et *al*., 2005). Despite the nutritional value of dried salted fish and fermented artisanal transformation processes pose safety problems related to the development of salt-tolerant bacteria (halophiles), fungi and parasites (Michel, 2005; Brigitte et *al*., 2005). The risks can be serious and are a growing problem for public health (OMS, 2002). In many countries, there have been in recent decades significant increases in the incidence of infections caused by microorganisms transmitted primarily through food, including fish (Aoued et *al*., 2010a; 2010b) diseases. This study aims to assess the hygienic quality of dried salted fish and fermented marketed in the town of Lomé-Togo.

2. Materials and Methods

The work was carried out in Laboratory of Microbiology and Quality Control of Foodstuffs, Hight School of Biological and Food Techniques (ESTBA) of University of Lomé, Togo.

2.1 Study material

It consists of salt-dried fish and fermented fish taken within two (2) markets in Lome. These markets are "Dowevikpanou and Atikpodji".

2.2 Methodology

2.2.1 Sampling

The samples were collected in sampling required conditions. The samples were then wrapped in aluminum paper and fed into a cooler before being transported to the laboratory for microbiological analyzes.

2.2.2 Preparation of the stock solution

The stock solution is obtained by grinding 25 g of fish samples with 225ml of buffered peptone water. This produces a dilution of 1/10. The stock solution obtained is left at room temperature about 45 mn to 1 hour for revivification. Then, a series of decimal dilutions are made from the stock solution for the enumeration of microorganisms.

2.2.3 Research and counts germs

Germs sought were those used by AFNOR (French Association for Standardization) criteria in Article 10 of Decree No. 1391 / CM 23 October 1998, to be met by salted and smoked fish that we have adopted for fermented fish. These are: aerobic mesophilic flora (NF.V08-051); Total coliforms (30 ° C NF.V08-050); *Staphylococcus aureus* (37°C) (NF.V08-057-1); *Salmonella sp.* (37 °C) (AFNOR.V08-052). We also searched for information, yeasts and molds (NF.V08-075), thermotolerant coliforms and *E. coli* (44 °C) (EN ISO 16140); and sulphite-reducing anaerobes (44 °C) (NF.V08-061), not considered by the criteria. After counting, we collected two (2) colonies for all samples in the totals positive smear preparation germs to assess bacterial flora of dried salted fish and fermented fish. On the same germs, we also conducted the testing guidelines (catalase and oxidase). Then, these strains were maintained on the nutrient medium in the tube.

The results of the microbiological analysis are in Unit Forming colonies per gram (CFU/g), and then analyzed in relation to those criteria in Article 10 of October 23, 1998. We also considered the analytical variability associated with of methods analysis for the plan to three (3) classes. The results are also analyzed by the Epi-info software dfr- 64 April 2001 for the comparison.

Tableau 1 : Appreciation of the hygienic quality of dried salted fish,taken in the two markets Dowevikpanou and Atikpodji

Germs	Average n=15 (CFU/g)	Extrem value (CFU/g)	Criteria			Hygienic Quality (%)		
			m*	3m*	10m*	Satisfying	acceptable	Unsatisfactory
Total Germs (30°C)	$2.52x10^6$	$0.00007x10^6$ $27x4.\ 10^6$	10^6	$3x10^6$	$30x10^6$	73.33	13.33	13.33
Total Coliform (30°C)	0	0	1	3	30	100	0	0
Coliform thermotolerant (44°C)	0	0	NC	NC	NC	NA	NA	NA
E. Coli	0	0	NC	NC	NC	NA	NA	NA
Sulfito-reducing anaerobes (44°C)	5.71	0-50	1	3	30	80	0	20
S. aureus	10	0-80	5	15	50	80	6.66	13.33
Salmonella sp.	0	0	Ab/ 25g NC	Ab/ 25g	Ab/ 25g NC	100	0	0
Yeast and Mold (30°)	0	0		NC		NA	NA	NA

n = Total number of samples; m = Standards AFNOR (French Agency for Standardization) *Acceptability limit related to the method of counting : 3 m*= Solid medium; 10 m* = Liquid medium ; NA = Not applicable; NC: Not considered

The results of these analyzes are shown in Table 1. Microbial testing is outside the presence of total coliforms or thermotolerant coliforms and *E. coli* or *Salmonella sp.*, or yeasts and molds. Mesophilic aerobic bacteria (30 °C) are found in all samples. Values range are between $0.00007x10^6$ CFU / g and $27.4x\ 10^6$ CFU/g, with an average of $2.52x10^6$ CFU/g. The sulphite-reducing anaerobes (44 °C) were found in four (4) samples with rates ranging from 0 to 50 CFU / g of product with an average value of 5.71 CFU / g. The observed values are higher than those set by the criteria. The enumeration of *S. aureus* in three (3) samples gave high values of the order of 0 to 80 CFU / g, with an average of 10 CFU / g. The values are higher than those set by the criteria.

Tableau 2: Findings of the hygienic quality of fermented fish, taken in two markets "Dowevikpanou and Atikpodji".

Germs	Average n=15 (UFC/g)	Extrem Value (UFC/g)	Criteria			Hygienic Quality (%)		
			m*	3 m*	10m*	Satisfying	acceptable	Unsatisfactory
Total Germs (30°C)	$0.52x10^6$	$0.0001x10^6$ $2.15x10^6$	10^6	$3x10^6$	$30x10^6$	73.33	26.66	0
Total Coliform (30°C)	1.26	0-19	1	3	30	93.33	0	6.66
Coliform thermotolerant (44°C)	0	0	NC	NC	NC	NA	NA	NA
E. Coli	0	0	NC	NC	NC	NA	NA	NA
Sulfito-reducing anaerobes (44°C)	4	0-20	1	3	30	73.33	0	26.66
S. aureus	0	0	5	15	50	100	0	0
Salmonella sp.	0	0	Ab/ 25g	Ab/ 25g	Ab/ 25g	100	0	0
Levures et moisissures (30°C)	0	0	NC	NC	NC	NA	NA	NA

n = Total number of samples; m = Standards AFNOR (French Agency for Standardization) *Acceptability limit related to the method of counting: 3 m* = Solid medium; 10 m* = Liquid medium ; NA = Not applicable; NC: Not considered

The results of these analyzes are shown in Table 2. Fermented fish analyzed contain no thermotolerant coliforms and *E. coli* or *S. aureus*, *Salmonella sp.*, or yeasts and molds. Mesophilic aerobic germs (30 °C) are present in all samples, with values lying between $0.00071x10^6$ and $1.41x10^6$ CFU / g and an average of $0.52x10^6$ CFU / g products. The values obtained in four (4) samples analyzed have a level located beyond the values set by the criteria. Total coliforms were counted in a single sample (19 CFU / g). This value is higher than that set by the criteria. The sulphite-reducing anaerobic bacteria were found in five (5) samples, or their extreme values are 0-20 CFU / g, with an average of 3.33 CFU / g. These values are higher than those set by the criteria. It is clear from our results that 73.33% of these products are of satisfactory hygienic compared to total bacteria; while 93.33% are acceptable in relation to total coliform and 73.33% from the sulphite-reducing anaerobes. Regarding the *S. aureus* and *Salmonella sp.*, all products are of satisfactory hygienic compared to the thresholds of the criteria.

Table 3: Evaluation of non-compliance of commodities analyzed

N°	Germs	Percentage of non-compliance	
		Dried salted fish	Fermented fish
01	Total Germs (30°C)	26.66	26.66
02	Total Coliform (30°c)	0	6.66
03	Coliform thermotolerant (44°C)	NC	NC
04	*E. Coli*	NC	NC
05	Sulfito-reducing anaerobes (44°C)	20	26.66
06	*S. aureus* (37°C)	13.33	0
07	*Salmonella sp.*	0	0
08	Yeast and Mold (30°C)	NC	NC

NC = Not considered

The analysis of these products indicated in Table 3, did not show the presence of coliforms, thermotolerant *or E. coli*, *Salmonella sp.*, or yeasts and molds. The total bacteria, anaerobic sulfite-reducing and *Staphylococcus aureus* are the bacteria most implicated in the contamination of dried salted fish and fermented analyzed. Total coliforms were found in a single sample of fermented products. Total seeds and sulphite-reducing anaerobes, are most often implicated in the microbial contamination of these products germs. The non-compliance rate induced by the total germs is 26.66% in

both types of fish. About sulfite-reducing anaerobes, we noted 26.66% of non compliance in fermented fish, and 20% in dried salted fish. Total coliforms have induced a non-compliance rate of 6.66% in fermented fish. *S. aureus* (pathogens) have a rate of induced non-compliance of about 20% in dried salted fish. These germs are not present in fermented fish analyzed.

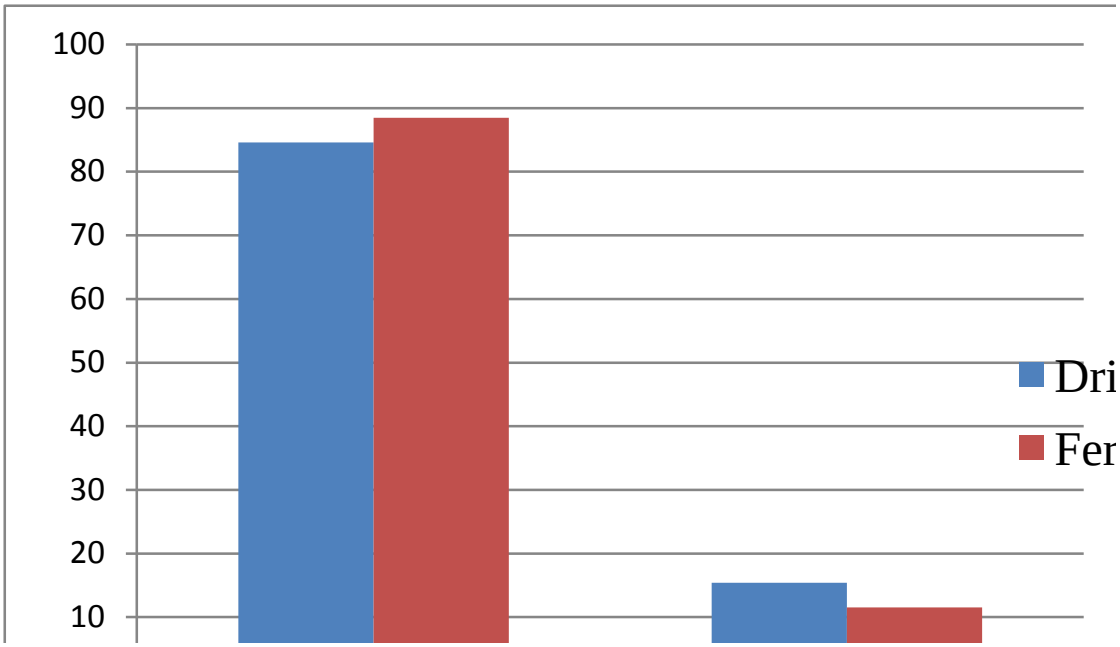

Figure 1 : Evaluation of microbial flora isolated from dried salted fish and fermented fish

We have sought to see the types of bacteria that colonize dried salted fish and fermented fish with salt concentration is high (16-20%). However, the results of the evaluation performed of the microbial flora from Total bacteria showed the presence of Gram positive bacilli to a percentage of 84.61% against 15.38% of Gram positive cocci at dried salted fish (for 26 germs isolated). Moreover, 88.46% of Gram positive bacilli against 11.53% of Gram positive cocci, have been isolated from fermented fish (for 26 germs isolated).

3. Discussion

Appreciation of the hygienic quality of dried salted fish, taken in the two markets "Dowevikpanou and Atikpodji". It is clear from our analysis that 73.33% of the samples (dried salted fish and fermented fish) are satisfactory hygienic compared to total bacteria, while 80% (dried salted fish) and 73.33 % (fermented fish) are satisfactory too with sulphite-reducing anaerobes. However, 13.33% of the products (dried salted fish) are of unsatisfactory hygienic compared to *S. aureus*. In addition all products are of satisfactory sanitary quality compared to total coliforms and *salmonella sp*. The presence of *S. aureus*, often involved in poisoning, is the failure to observe the rules of good practice preparation. This confirms the work of Anouhivi et *al*. (2005, 2006 and 2007), they note that insufficient salting could lead to the proliferation of undesirable microorganisms such as *S. aureus*. Literature of Momar (2007) argues that the salts may contain many salt-tolerant bacteria (halophiles), including *S. aureus* and some strains produce enterotoxin and cause food poisoning. Furthermore, the presence of sulphite-reducing anaerobes, telluric germs, may suspect the presence of *Clostridium perfringens*, pathogenic species responsible for food poisoning. Despite the high concentration of salt, fish often undergo microbial and / or insect infestation contamination during processing, storage and marketing (Zakhia, 2000).

3.1 Findings of the hygienic quality of fermented fish, taken in two market "Dowevikpanou and Atikpodji"

The presence of sulfite-reducing anaerobes in a product, on the one hand indicate a breach of the rules of hygiene and also a risk of food poisoning by *Clostridium perfringens* such as. According to Michel (2005), the sea water used as an ingredient in some traditional fermentation processes can be a potential source of bacterial pathogens including *Clostridium sp.* and *Vibrio sp*. Indeed, total coliforms found in fermented fish, does not necessarily reflect a faecal contamination. But their presence and that *Escherichia coli* indicate a certain faecal contamination. Same observation finding by Abbey et *al*. (1994), in the "Momone" fermented fish in Ghana. This author says that faecal contamination resulting from the simultaneous presence of total coliforms, thermotolerant coliforms and *E. coli* in these products. According to Guiraud (1998), coliforms are generally not dangerous from the health point of view, except in cases of extremely abundant proliferation or receptivity of the consumer. In Cameroon, Baba (1985), obtained in fermented dried fish $123x10^3$ CFU / g of total viable flora and $35x10^2$ CFU / g of total coliforms. In Benin, the work done by Anihouvi et *al*. (2005, 2006 and 2007), on the "Lanhouin" reported that 40% of products are of acceptable quality compared with total viable flora, and total coliforms , and *Clostridium sp* are listed little (6%) of samples. According to these authors, the presence of *Clostridium sp*, even at a very low number, needs to improve the handling and manufacturing processes thereof. The absence of pathogens like *Salmonella sp.*, *S. aureus*, yeast and mold can be explained by the high concentration of salt in products. Although *S. aureus* germs are salt-tolerant; they cannot tolerate a concentration greater

than 17% salt (Codex Alimentarius, 2005); while the salt concentration of these products is of the order of 15-25% of the mass of fish.

3.2 Evaluation of non-compliance and origin of contamination of the food analyzed

The presence of total germs, sulphite-reducing anaerobes and *S. aureus* and to a lesser extent total coliform is due to breaches of hygiene rules during production, transportation, storage, or handling. It is clear from the evaluation that dried salted fish is better hygienic than fish fermented over total coliforms. As against, fermented fish, are better hygienic qualities than dried salted fish relative to *S. aureus*. But statistically: There would be no significant difference between dried salted fish and fermented compared to total bacteria ($p = 0.31 > 0.05$), and sulfite-reducing anaerobes ($p = 0.54 > 0.05$).

3.3 Evaluation of microbial flora isolated from dried salted fish and fermented

Indeed, the predominantly Gram positive bacilli in the fermented fish, shows that the fish is a good breeding ground due to the presence of the protein which is a good substrate that supports their development. Similar results were obtained by Azokpota (2006), on the fermentation of grain of "Néré" for the production the of "Afitin", these results showed that the protein is a good substrate for alkaline fermentation, by hydrolysis into peptides and amino acids, resulting in an increase of the pH, creating a hostile environment for microorganisms contaminants and allows storage of the fermented product for a long period. Other results for the microbial flora of fermented fish, have been obtained by some authors, in Ghana on "Momone" by Nerquaye-Tetteh et *al*. (1978); Abbey et *al*. (1994), and in Benin on the "Lanhouin" by Anihouvi et *al*. (2006 and 2007). They indicate that fermented microbial poisons are dominated by Gram-positive bacilli, such as *Bacillus sp*., and a few cocci including : *S. aureus* and micrococcus. In addition to these germs, Koffi-Nevry et *al*. (2011) in Cote d'Ivoire have isolated in "Adjuevan" fish fermented lactic acid bacteria such as *Leuconostoc lactis, Lactobacillus fermentum, Pediococcus sp.*, Streptococcus, which are all gram positive bacteria.

Also, it appears from this study that there are deficiencies in hygiene practices in the handling of these products. Several other authors (Barro et *al*., 2006; Abdelrahim et *al*., 2012; Tidjani et *al*., 2013a; Tidjani et *al*., 2013b) reported in their studies failure to comply with basic rules of hygiene in production and marketing of food.

4. Conclusion

This work has allowed us, firstly to assess the hygienic quality of dried salted fish and secondly that of fermented fish. Our results showed that dried salted fish and fermented fish analyzed are free of *Salmonella sp*, thermotolerant coliforms, *Escherichia coli*, yeast and molds. In dried salted fish, the rate of non-compliance identified by the criteria is 26.66% for the total bacteria (30°C), 20% for sulphite-reducing anaerobes, and 20% for *Staphylococcus aureus.* All products are of satisfactory hygienic quality vis-à-vis total coliforms. On fermented fish, the rate is 26.66% and 6.66% respectively compared to total bacteria (30 °C) and total coliforms (30 °C) and 26.66% compared with sulphite-reducing anaerobes. None of these samples containing *S. aureus*. In addition, dried salted fish is better hygienic than fish fermented over total coliforms. And fermented fish, is also more hygienic than dried salted fish compared to *S. aureus* qualities. However, the results of the evaluation performed of the microbial flora from Total bacteria showed the presence of Gram positive bacilli to a percentage of 84.61% against 15.38% of Gram positive cocci in dried salted fish (for 26 germs isolated). As against, 88.46% of Gram positive bacilli, and 11.53% of gram positive cocci, have been isolated from fermented fish. The majority of Gram-positive are positive catalase and negative oxidases. Cocci are catalase and oxidase positive. The isolated bacteria are retained in order to perform their molecular characterization.

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