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Evaluation of the Microbiological Quality of Smoked Fish Taken at Lake Ahémé of Benin

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ABSTRACT

The present study aims to evaluate the microbiological quality of smoked fish from some lake villages near Lake Ahémé. To do this, samples of smoked fish were taken at different sites, followed by microbiological analyzes. The results obtained after the surveys showed, two technological variants of smoking that the fish is lean or fat. Those resulting from the evaluation of the microbiological quality of the fish reveal the presence of several microorganisms including total mesophilic aerobic flora, total coliforms, staphylococci with maximum values of 320.102 cfu / g for coliforms and 280.102 cfu / g for Staphylococci exceeding the normative criteria. It also showed the presence of the fungal flora, the complete absence of fecal coliforms and sulphite-reducing anaerobes in all the samples studied.

Introduction

The substantial contribution that oceans and inland waters can make to the adequate food and nutrition security of the world's population is increasingly recognized (FAO, 2016). Fish and fish derivatives contribute around 60% of the world protein supply and more than 30% of the animal protein intake of developing countries (FAO, 2000). Fish production in Benin does not cover domestic demand. For an annual requirement of about 200 000 tonnes, the national average annual production of fish, fish and aquaculture combined, is estimated at about 45 000 tonnes, a deficit of 155 000 tonnes, partly filled by the import of frozen fish about 75 800 tonnes (FAO, 2006). These production and consumption deficits are aggravated by post-harvest losses linked to the very high perishability of fresh fish. In Benin and other countries in the sub-region, post-harvest losses are estimated at around 20% (Anihouvi & *al.*, 2005).

To limit post-harvest losses, various preservation methods such as frying, drying, salting and smoking are used individually or in combination (Yacoubou 2009; Issa & *al.*, 2012). These methods of conservation aim not only to prevent the proliferation of the micro-organisms of deterioration, but also to slow certain biochemical reactions (ANSES, 2010). Traditional smoking is one of the main methods of fish conservation practiced by the populations of villages near Lake Ahémé in southwestern Benin. Unfortunately, the artisanal character of fish smoking, the lack of hygiene in production greatly favor microbial contamination of products (Abochi, 2010). In addition, traditional methods of keeping fish after smoking favor the development of other pathogens (Djinou, 2001). In general, the production and sale of smoked and smoked-dried fish are made in an unsanitary environment, without respecting good hygiene and manufacturing practices. Indeed, the fish are washed with water of dubious quality, sold in the open, without protection, sometimes in unhygienic environments with the remarkable presence of flies. A recent study conducted in Benin on the microbiological quality of smoked and smoked-dried fish in the artisanal sector revealed a high contamination marked by the presence of *Enterobacteriaceae* (80%), Escherichia coli (17%), *Bacillus cereus* (77%) , *Clostridium perfringens* (72%), yeast and mold (100%) in the samples collected (Covo, 2017). Thus, the contaminated fish obtained can be at the origin of food poisoning and gastric diseases. In fact, foods that are unfit for consumption and contain bacteria in particular cause more than 200 diseases, ranging from diarrhea to cancer (WHO, 2017). Despite these potential risks and the high production of smoked fish in the riparian villages of Lake Ahémé, few studies have been conducted to determine the sanitary quality of smoked fish in this locality.

It is therefore important to evaluate the microbiological quality of smoked fish in some villages near Lake Ahémé.

Materials and methods

Sampling of fish species

Nine fish species were collected in the villages of Bopa, Topka Domè and Ouèdèmè namely : Ray (*Manta birostris*) Wetin (*Liza falcipinis*), white Sillure (*Silurus Linnaeus*) Gban (*Elops lacerta valvercienne*), catfish (*Arius africanus*), Fretin (*Ethmalosa finbriata*), Catfish (*Ameiurus melas*), Tilapia (*Oreochromis alcalia*) and Kpankpan (*Caranx latus*). The choice of sites was based on the number of transformers since each processor at her own smoking site. Sampling of fish samples for microbiological analyzes was performed under aseptic conditions: sterile latex gloves are used for hand protection during sampling; fish samples are collected and packaged in sterile bags and packaged in a portable ESKIMO cooler. All sampling equipment is previously sterilized with cotton soaked in alcohol at 90 ° C.

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Physico-chemical analyzes :

- **Determination of pH**

5g of fish were crushed and then dissolved in 25ml of distilled water. The mixture was filtered on Wattman paper. 20 ml of this solution are taken from a beaker in order to read the pH after having calibrated the pH meter with the pH 4 and 7 buffer solutions (Nout & *al.*, 1989).

- **Dry matter content**

The determination of the dry matter content is carried out according to the method described by Ezoua et al. (1999). For this fact, 5g of smoked fish was taken from an aluminum bowl and initially tared. Then the whole was heated in an oven for 24 hours at 105 ° C. After cooling in a desiccator, the dehydrated test portion is weighed again. The dry matter content of the fish was determined by the formula :

$$\%MS=\frac{m2-m0}{m1-m0}\times 100$$

Is :

m0: mass of the bowl

m1: mass of the bowl with the wet sample

m2: mass of the bowl with the dried sample

Microbiological analyzes

The samples taken were evaluated by searching by standard methods reported by Joffin and Joffin (2003) quality microbiological parameters. These are total mesophilic flora at 30 ° C (total germs, NF V08-051), total coliforms, thermotolerant coliforms and *Escherichia coli* (NF ISO 4831) of Staphylococci spp at 37 ° C (NF EN ISO 6888). -1), *Salmonellae* (NF V 08 - 052), yeasts and molds (ISO 7954) and spores of Anaerobic sulphito-reducers (NF V 08-061). The culture media and reagents used come from Laboratoires BioMérieux and Diagnostics Pasteur. The interpretation of the results was made according to a two-class plan with reference to the microbiological criteria for fresh animal products (French legislative and regulatory guide, No. 8155 of 12 December 2000), setting the tolerance threshold at M = 103 CFU / g or ml for total flora; at 10 CFU / g or ml for faecal coliforms; 2 CFU / g or ml for sulphite-reducing bacilli and the absence in 25 g of product analyzed for salmonella.

- **Preparation of the mother suspension**

25 g of each sample were removed and aseptically ground. 225 ml of buffered peptone water were added to the mash and the mixture was homogenized with stomacher. From this suspension, decimal dilutions were carried out.

- **Total flora count**: It was carried out by seeding in the mass. One (1) ml of the stock suspension and its decimal dilutions in duplicate were seeded in the Plate Count Agar (PCA) Supercooling Agar. The incubation was carried out at 30 ° C. for 72 h, then the count and the average of the colony-forming unit (CFU) / g of sample analyzed were made according to the method specified by the NF V08-051 standard.
- **Yeast and Mold Count**: 0.1 ml aliquots of the stock suspension and its decimal dilutions were surface seeded on PDA agar thoroughly prepared and cast in 9 cm diameter dish. The enumeration of white or colored colonies, smooth and creamy yeast and powdery mold was performed after 5 days of incubation at 25 ° C according to ISO 7954.
- **Search for total and faecal coliforms**: Total coliforms are searched according to the MPN method described by standard NF ISO 4831. The search for thermotolerant coliforms is carried out by counting colonies obtained at 44 ° C according to the method specified by standard NF V 08 -060.
- **Staphylococci Investigation**: The surface spread technique of 0.1ml inoculum (sample and decimal dilutions) on Baird Parker medium was used. Incubation of the inoculated media was done at 37 ° C / 48H. The method used is that described by standard NF EN ISO 6888-1.
- **Research of anaerobic spheroids**: The search for spores of Anaerobes Sulphito-reducers makes it possible to evaluate the risks of Clostridium spp. The culture medium used is Tryptone Sulfite Neomycin (TSN). The method used is the anaerobiosis enumeration of sulphite-reducing bacteria described by standard NF V 08-061.
- **Salmonella Research**: Salmonella research in foods includes essential steps such as pre-enrichment, enrichment, isolation and confirmation. The method used for this research is that specified by standard NF V 08-052.

Statistical analyzes

The results were analyzed by the variance method (ANOVA) using STATISTICA software (Stat., Soft, Inc., 1995). The comparison of the averages is performed by the test of the smallest significant difference LSD (Least Significant Difference). This method of analysis consists of looking for averages that differ significantly from one another. The differences are significant when $P < 0.05$.

Results and DIscussion

The results of the semi-structured surveys carried out at the different smoking sites investigated showed that all of the transformers originated from the locality and were passed on the art of smoking their parents' fish. They are uneducated and only have fish smoking as their only income generating activity. These results made it possible to list nine different species of fish, namely: Ray (*Manta birostris*), Wetin (*Liza falcipinis*), White Sillure (*Silurus linnaeus*), Gban (*Valvercian Elops lacerta*), Mâchoiron (*Arius africanus*), Fretin (*Ethmalosa finbriata*), Catfish (*Ameiurus melas*), Tilapia (*Oreochromis alcalia*), Kpankpan (*Caranx latus*). The smoked fish are directly from fishing so fresh. Their smoking is done with all types of wood and they are marketed directly around smoking sites.

Depending on the type of fish, there are two smoking technologies. The first in which the fish is subjected to a large fire to reduce its moisture content before being subjected to smoke is used for lean fish such as Fretin to ensure a long shelf life ranging from 2 to 6 weeks. Regarding the second variant it is used for oily fish such as skate, catfish and others. The fish are subjected to the simultaneous action of smoke and fire for a 2 to 6 hours of time depending on the species of fish. The shelf life of these is 2 to 3 days at most. To ensure their preservation and avoid putrefaction, processors heat up and package using cement paper and baskets every night after the market if all their stocks of smoked fat fish are not finished.

The production diagrams for lean and fat smoked fish were presented in Figures 1 and 2 respectively.

The physical appearance of the different types of smoked fish encountered at the sales sites in the study area was presented in Photo 1.

Fresh fish showed a variation in color depending on the species. At the time of capture they showed a generally firm texture except for those dead in the water before capture. Smoked fish, on the other hand, have a totally golden color, which also varies according to the species of fish; the flesh is totally tender for oily fish and totally dry for lean fish. This color presented by the fish at the end of smoking is due to the smoke used in the smoking process. Indeed, according to the work of Talon and Girard (1980), the smoke consists of a suspension of solid and liquid particles in a gaseous medium; the substances contained in these phases are the same, but in

different concentration. The liquid phase represents about 90% of the smoke. Its particles measure about 0.1 micron, are poorly soluble and have high boiling points. The most volatile chemical substances, which are absorbed by fish, are mainly in the vapor phase. They dissolve in the superficial water of the fish. Droplets or particulate phases do not play an essential role in the smoking process, but serve rather as a reservoir for constituents for the gas phase. The equilibrium between the two phases can be modified by the temperature and by the admission of air; the proportion of solid and liquid particles in the gaseous medium determines the density of smoke.

Photo 1. Physical appearance of fish before and after smoking

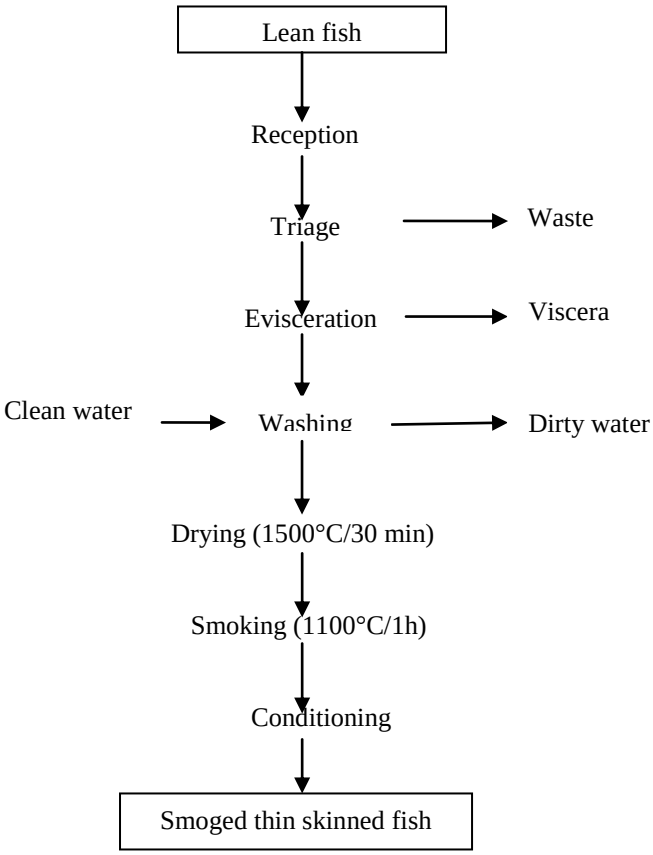

Figure 1 . Diagramme technologique de production de poisson maigre fumé conditionné

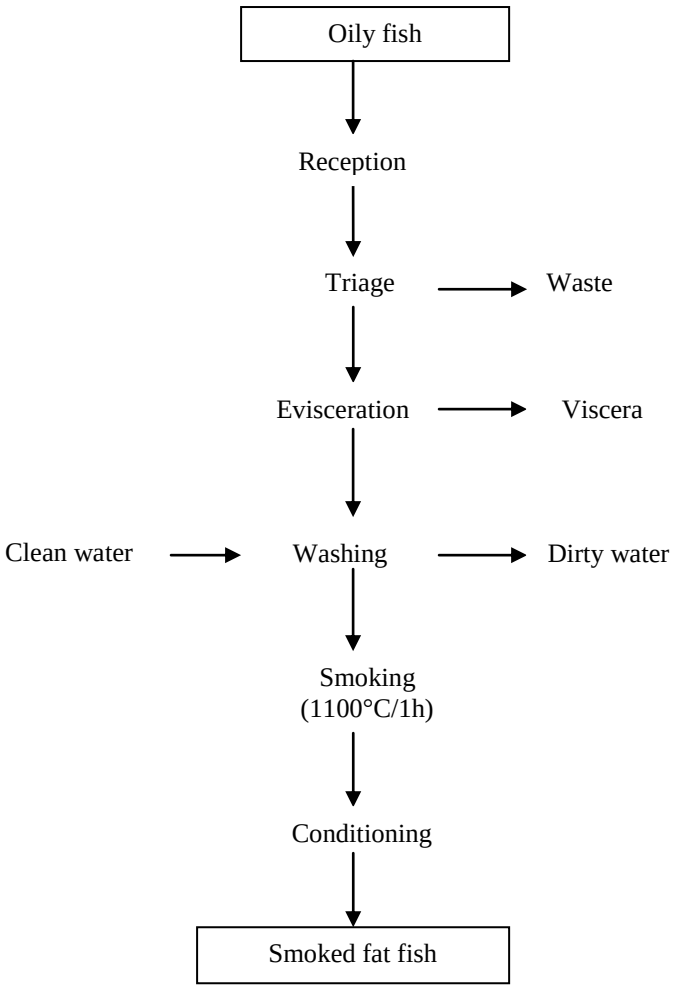

Figure 2 . Diagram of production of smoked fat fish

The results of the determination of dry matter and pH of the samples were presented in Figures 3 and 4.

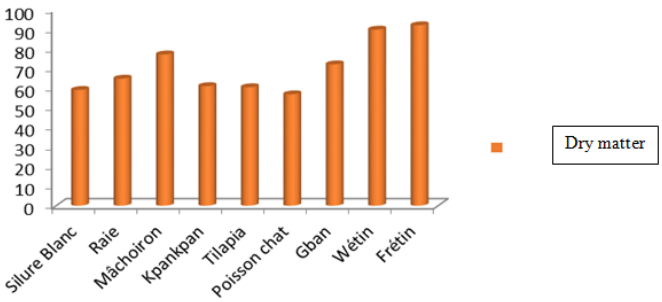

Figure 3 . Variation of the material Dry matter

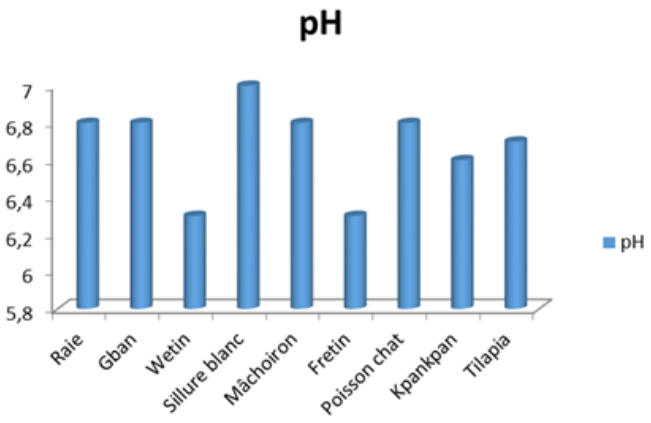

Figure 4 . Variation of the pH of the samples

Catfish had the lowest dry matter content (56.77%), whereas the small size fry had the highest dry matter content (91.96%). These results also show that the dry matter content of fish is highly dependent on the type of fish and the smoking technology used. Indeed, this variation in the dry matter content is related to the water content of the different fish species used in this study as well as to the technology used. The pH results showed that the sample of white catfish at the highest pH (7) while that of fry at the lowest pH (6.3). It should be noted that all pHs are close to neutrality. This neutral pH could be detrimental to the microbiological quality of the different fish samples.

In Table I were presented the results of microbiological analyzes carried out on the various samples of smoked fish collected in the villages bordering Lake Ahémé according to the type of germ sought.

Aerobic total mesophilic flora is present in all samples. The results obtained are close to those reported by Faton (2014) who worked on frozen fish smoked in Djidje and Sainte Cecile (Cotonou). The enumeration of total flora is useful in that it can be used to define deviations from good manufacturing practices, including delays in product development, (Ababouch, 1995). Its presence in large numbers indicates the alteration of the product. These germs do not have a great impact on the health of the consumer, on the other hand they could cause significant economic losses because of the alteration of the products.

The results obtained during the search for faecal coliforms showed a total absence in all the samples studied. However, with the exception of fish species such as wetin, tilapia, white catfish, fretin and gban, Bopa wetin exhibited the largest microbial load of total coliforms to know 280.102 cfu/g while the Fretin sample Bopa had the lowest contamination rate of 2.102 cfu / g. These results characterize the unsatisfactory quality of all the fish species mentioned. The results thus obtained differ from those of Abochi (2010) who evaluated the microbiological quality of fish smoked by hand in Togo. The low rate of total coliform contamination and the absence of faecal coliforms in the samples would be an indicator of the good hygiene of the processors since coliforms are vectors of recent faecal contamination. The presence of thermotolerant coliforms in a food is indicative of poor hygiene conditions, in this case personnel hygiene. Indeed, they are the host of the digestive tract of humans and animals. Their presence is due to faecal contamination. Smoking workshops do not have a device for washing and disinfecting hands. Thus, the requirement to wash one's hands before each return to work is not observed. In addition, some of these workshops are on the outskirts of the Cotonou-Lomé State Interstate, wandering animals could, through their faeces that they leave during their passage on smoking sites, contaminate smoking equipment and products.

Microbiological results also showed the presence of *Staphylococci spp* in all samples analyzed. The microbial loads of all smoked staphylococcus spp. Fish ranged from 19.22 cfu / g for the Ouedema tilapia sample to 320.102 cfu / g for the Bopa chickweed sample. The values obtained are higher than the norm, with a compliance rate equal to 0%. The results thus obtained are different from those obtained by Faton (2014) who detected the presence of staph in only some of his samples. Since staphylococci are indicators of cutaneous contamination, their presence could be explained by the fact that the manipulators (depending on the level in the smoking and distribution chain) are suffering from *Staphylococcal rhinopharyngitis*, angina, sinusitis or lesions. infected hands and did not protect their hands before handling smoked fish. The results obtained are close to those of Wade (1992) which is 2.56.104 cfu, but much higher than those of Khallaf et al. (2003) who obtained a microbial load of 468 cfu / g. *Staphylococcus aureus* is the cause of food poisoning. Indeed, this germ is carried by many subjects on the surface of the skin and mucous membranes. According to Buyser

Table I. Results of microbiological analyzes

Sittings / Samples		Total flora (cfu/g)	Coliforms		Staphylococcus *Spp* (cfu/g)	Sulfite-reducing anaerobic (cfu/g)	Fungal flora	
			Totals (cfu/g)	Fecal (cfu/g)			Yeast (cfu/g)	Mold (cfu/g)
B	Raie	>300	Absence	Absence	20.10^2	Absence	Présence	Absence
	Silure Blanc	>300	129.10^2	Absence	81.10^2	Absence	Présence	Absence
	Tilapia	>300	31.10^2	Absence	122.10^2	1	Presence	1
	Wètin	>300	280.10^2	Absence	46.10^2	Absence	Presence	Absence
	Fretin	>300	2.10^2	Absence	$35.\ 10^2$	Absence	Presence	Absence
	Mâchoiron	>300	Absence	Absence	320.10^2	Absence	Presence	Absence
	Poisson chat	>300	Absence	Absence	56.10^2	Absence	Presence	2
T	Tilapia	>300	Absence	Absence	21.10^2	Absence	Presence	Absence
	Gban	>300	5.10^2	Absence	59.10^2	Absence	Presence	Absence
	Kpankpan	>300	Absence	Absence	67.10^2	Absence	Presence	Absence
O	Tilapia	>300	Absence	Absence	$19.\ 10^2$	Absence	Presence	1
Microbiological criterion (AFNOR 1996)		10^6	Absence	Absence	1	Absence	ND	ND

Legend **: Bopa, Tokpa-domè, Ouèdèmè**

(1980) cited by Sakho (1988), the importance of the role of the carriers of germs (suppurative skin wounds) in the contamination of food by *Staphylococcus aureus* is not to be underlined any more. About 30% of subjects would be carriers. Thus, *S. aureus* can be an indicator of human contamination, from the nasopharyngeal mucosa and skin. Prophylaxis of *S. aureus* poisoning should be involved, although the risk of poisoning in the case of our samples is low because of the cooking method used before consumption.

As for the determination of Sulfite-Reducing Anaerobes, only the tilapia sample taken from Bopa is contaminated. The results thus obtained are 95.66% in accordance with the standard of 0 cfu / g and are different from those of Abochi, 2010 which had a contamination threshold of 3.75% in its samples of smoked fish sampled. in Togo. These germs secrete enterotoxins responsible for serious toxi-infection. The search for *Salmonella spp* at the level of the different samples from different villages shows the total absence of contamination by Salmonella. These results show that the samples are satisfactory. *Salmonella spp* being a very dangerous germ responsible for typhoid fever, its presence in the samples would have raised great questions. *Salmonella enteritidis* is the serotype typically present in the reproductive tract of animals (De Buck *&* ***al.***, 2004). Other serotypes such as *Salmonella typhimurium* are more common in broilers and to a lesser extent in other meat production chains. Pork meat to a lesser extent and beef are the other most common sources of contamination. Plant products can also be a source of Salmonella due to the use of contaminated water or fertilizers (De Buck *&* ***al***., 2004).

The results of the determination of the fungal flora show the presence of yeast in all the samples studied. However molds are only found in two samples, namely Bopa tilapia and Tokpa-domè kpankpan. The fungal flora being an indicator of the commercial quality of smoked fish, its presence is attributable to the exposure of fish after smoking to dust.

Conclusion

The present study, carried out with the aim of assessing the microbiological quality of smoked fish in some villages around Lake Ahémé, first identified, following the investigation phase, two technological variant of fish smoking in function that the fish be lean or fat. Secondly, it showed the presence of several microorganisms, including total mesophilic aerobic flora, total coliforms and staphylococci in proportions exceeding the normative criteria. It also showed the presence of fungal flora in all the samples studied. However, it is important to note the total absence of fecal coliforms and sulphite-reducing anaerobes in all the samples studied.

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