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Occurrence of Microbial Loads in Smoked Fishes Marketed in the Lakeside Village of Guezin (Southern Benin) and Associated Microbiological Hazards

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Abstract Fish is highly nutritious with high protein content. However, it is a suitable medium for growth of microorganisms, if poorly processed. This study reports the occurrence of microbial loads in smoked fishes marketed in the lakeside village of Guezin (Southern Benin) and associated microbiological hazards. The results obtained indicated that the fish smoking activity in the lakeside village, is exclusively done by women (100%), in households and an informal situation, using traditional equipment. A total of thirteen species of fish, such as *Manta birostris*, *Marcusenius Senegalensis*, *Liza falcipinis*, *Hydrocynus brebis*, *Elops lacerta valvercienne*, *Silurus linnaeus*, *Silurus glanis*, *Arius africanus*, *Heterotis niloticus*, *Strongylura senegalensis valenciennes*, *Ethmalosa finbriata*, *Gymnocranius griseus* and *Ameiurus melas* are mostly smoked in this area. Microbial loads determined by using standard microbiological procedures, underlined the contamination of smoked fishes by microorganisms, with a high occurrence of Aspergillus strains (83.31%). This occurrence of fungi species could be due to absorption of moisture during storage, which could supported the growth of the microorganisms, in addition to the contamination during processing, handling and display on the market stall. It is then recommended that fish processors should ensure that fish products are properly hot smoked and dried so as to prevent fungal growth and mycological hazards.

Key words: *smoked fishes, lakeside village, microbial loads, mycological hazards, Benin*

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1. Introduction

Fish is one of the best supplies of proteins, vitamins and minerals and are essential nutrients for fortifying both infant and adult diets [1]. Fish is an important part of a healthy diet due to its high quality protein, other essential nutrients and omega 3-fatty acids, and its low fat content as compared to other meats [2]. Fish and seafood products constitute an important food commodity in the international trade due to its ever increasing consumption demand [3]. However, conservation of fish is became difficult due to the lack of adequate conservation system, and climatic and environmental conditions are favorable to its rapid degradation [4,5]. In West African country such as Benin, traditional conservation technics are currently used for the preservation of fresh fish [5]. Among them, smoking fish is an old traditional preservation technic which is transmitted from generation to generation. Nowadays, this endogenous technique is still practiced empirically by the producers, and under conditions that do not always guarantee the good microbiological quality of the smoked fish. Indeed, some bacteria are normally present on the fish which are associated with their natural environment and influenced by the season and harvesting conditions [6]. The proportion of the initial population can easily be changed after harvesting process depending on the ability of those bacteria to adapt to the new conditions [7,8]. Processing methods affect the microorganisms in fish in different ways, resulting in different types of micro-flora and different risks from spoilage organisms and pathogens.

It is generally accepted that fish with microbial load greater than10^6 cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption [9]. Then contaminated smoked fish could poses a serious health concern for consumers, due to the development of spoilage microorganisms. This contamination is also due to the climatic conditions, such as humidity which is in high level in lacustre village environment. In southern Benin, Guezin is one of the lacustre village characterized by an important fishing activity. This study, therefore aims to evaluate the occurrence of microbial load in smoked fishes marketed in the lacustre village of Guezin, as well as associated microbiological hazards. Findings will serve the purpose

of alerting consumers on the microbiological dangers of consuming poorly preserved smoked fish.

2. Material and Methods

2.1. Survey

A qualitative semi-structured survey was carried out in the main smoked fish production areas and the markets of the lacustre village of Guezin (Figure 1). A total, eighty (80) stakeholders of the sector, including producers (productions sites), traders and consumers (markets) were surveyed. The survey was carried out through individual interview by using a pre-established survey form. The searching information concerned: the socio-economic importance of the fish smoking activity in the village, the different species of fish commonly used, the different fish smoking technologies used and the post-smoking conservation methods of the fish.

2.2. Sampling of Smoked Fishes

A total of 60 samples of smoked fish were purchased from the different investigated sites. The sampling was performed under aseptic conditions: sterile latex gloves are used to protect hands during the sampling. Smoked fish samples are collected, packaged in sterile bags and put in a portable cooler. All sampling equipment is sterilized with alcohol at 90°C before their use.

2.3. Microbiological Analysis

For microbiological analysis, 25 g of each sample and 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72h. Desoxycholate was used for the total coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using Eosine methylene blue (EMB) medium. Tryptone sulfite neomycin agar was used for anaerobic sulfito-reducer (ASR) count, and tubes were incubated at 37°C for 24h. After incubation, the number of colonies was tracked, using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution. All media used for microbiological analysis were prepared as indicated by the manufacturer.

2.4. Fungal Isolation and Identification

The isolation of fungi from samples was performed using dilution plating method. 10 g of each smoked fish sample were added separately to 90 ml of sterile water containing, 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1}dilution. Further, 10fold serial dilutions up to 10^{-4} were made. 1 ml volume of each dilution were separately placed in Petri dishes, over which, 10 to 15 ml of potato dextrose agar amended with 60µg/ml chloramphenicol (PDAC) was poured. The plates were incubated at 28±2°C for 7 days. Fungal isolates from PDAC were sub-cultured on Sabouraud with chloramphenicol agar medium, and identification was carried out by using a taxonomic schemes primarily based on morphological characters, using the methods described by Singh et al. [10].

2.5. Statistical Analysis

Experiments were performed in triplicate, and data analyzed were mean subjected to one-way ANOVA. Means were separated by the Tukey's multiple range test when ANOVA was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).

Figure 1. Map of geographical situation of the lacustre village of Guezin

3. Results and Discussion

The results of the qualitative semi-structured survey carried out in fish smoking sites located at Guézin lakeside area have revealed that the fish smoking activity is exclusively done by women (100%). Fish smoking activity and the marketing of smoked fish are done in an informal situation. Producers are also limited by the unavailability of financial to acquire adequate smoking equipment. The low income of producers, combined with their low level of education, represent major constraints to this activity in the area investigated. Others surveyed indicated that this activity, however, allowed them to solve problems related to the living expenses and schooling of their children. The survey also revealed that the most fish smoking sites are located in households. The equipment used is still traditional. They are metal ovens made by local blacksmiths. It is a smokehouse made from a half-drum, equipped with a fireplace opening at the bottom and supporting a wire screen for the spreading of fish. Its surface is about 2 m², with a large fireplace opening of 0.51 m. Its nominal capacity is 16.27 kg of fish per load Figure 2.

Figure 2. Fish smoking equipment used in the lakeside village of Guezin

Regarding the different species of smoked fish used, the survey revealed that it depends on periods. However, thirteen (13) species of fish are mostly smoked in the study area. These are *Manta birostris*, *Marcusenius Senegalensis*, *Liza falcipinis*, *Hydrocynus brebis*, *Elops lacerta valvercienne*, *Silurus linnaeus*, *Silurus glanis*, *Arius africanus*, *Heterotis niloticus*, *Strongylura senegalensis valenciennes*, *Ethmalosa fimbriata*, *Gymnocranius griseus*, *Ameiurus melas*. For the post smoked fish conservation, the survey revealed that producers periodically smoked the unsold smoked fish in case of slacking, to avoid the risk of spoilage or putrefaction of smoked fish.

Table 1 indicated the results of microbiological analyses of smoked fish samples collected from lakeside village of Guézin. The results obtained underlined the high quantum of total flora in analyzed sample. The results obtained are close to those reported by Degnon et al. [11] in fish smoked in Cotonou (Benin). The presence of these microorganisms in high concentration in food product indicated the risk of rapid alteration of the product. These germs do not have a great impact on the health of the consumer, but they cause significant economic losses because of the alteration of products.

The low rate of total coliform contamination and the presence of *Staphylococcus spp* in the analyzed samples indicated the Low level of hygiene practices of women during the smoking process. Similar results were found on the other street foods. The germs most identified in these foods were mainly *Staphylococci* and enterobacteria. According FAO [12], epidemiological data in hospital showed a prevalence of 19% of diarrheal disease worldwide and bacterial diarrhea was estimated between 20 and 70% of the cases. The causes were related to poor hygiene found in the assessment of hazards and identification of critical points in the food processing chain [13].

Table 1. Results of microbiological analyses of smoked fish samples collected from lakeside city of Guezin

Fish species	Total flora	Coliformes		*Staphylococcus spp*	Anaerobic sulfito-reducer	Fungal flora	
		Total	Faecal			Yeast	mould
Manta birostris	>300	Absence	Absence	97.10^2	Absence	>300	Absence
Marcusenius Senegalensis	>300	Absence	Absence	53.10^2	Absence	>300	Absence
Liza falcipinis	>300	Absence	Absence	80.10^2	Absence	>300	5
Hydrocynus brebis guther	>300	Absence	Absence	25.10^2	Absence	>300	1
Elops lacerta valvercienne	>300	Absence	Absence	27.10^2	Absence	>300	Absence
Silurus linnaeus	>300	294.10^2	Absence	295.10^2	Absence	>300	2
Silurus glanis	>300	440.10^2	Absence	51.10^2	Absence	>300	Absence
Arius africanus	>300	Absence	Absence	54.10^2	Absence	>300	Absence
Heterotis niloticus	>300	Absence	Absence	12.10^2	Absence	>300	Absence
Strongylura senegalensis valenciennes	>300	Absence	Absence	105.10^2	Absence	>300	Absence
Ethmalosa fimbriata	>300	Absence	Absence	107.10^2	Absence	>300	Absence
Gymnocranius griseus	>300	Absence	Absence	49.10^2	Absence	>300	2
Ameiurus melas	>300	Absence	Absence	56.10^2	Absence	>300	2
Microbiological criteria (AFNOR 1996)	10^6	Absence	Absence	Absence	Absence	-	-
Conformity (%)	00	84,62	100	00	100	-	-

The results from the isolation and identification of fungi in pure culture have indicated the contamination of analyzed samples by fungi, with a high occurrence of *Aspergilla* strains (83.31%) (Table 2). This high contamination by *Aspergilla* strains could be in relation with the open-air exposure of products while they were displayed for sale. Indeed, several studies have reported the toxinogenic potential of some of *Aspergilla* strains isolated from food and foodstuffs in Benin [14,15]. These fungi can produce a significant amount of mycotoxins, when food storage conditions are inadequate. Review of literature on mycotoxin related human diseases, clearly reveals a linkage between ingesting of mycotoxin-contaminated food and illness, especially hepatic, gastro-intestinal, carcinogenic and teratogenic diseases [16]. Among these mycotoxins, Aflatoxins (AFs) produced predominantly by *Aspergillus parasiticus* and *A. flavus* are highly toxic as they are carcinogenic, teratogenic, and causing human liver and extra-hepatic cancers [17]. Other mycotoxins such as ochratoxin A (OTA) and citrinin (CIT) produced by *Aspergillus ochraceus* [18] are also detected in food. The co-contamination of fungi strains which can produce aflatoxins and ochratoxin A in smoked fish should be taken into consideration.

Table 2. Moulds isolated from smoking fish species

Mould species	Frequency (%)
Aspergillus ochraceus	16.66
Aspergillus wenti wehener	8.33
Aspergillus orizea	25.03
Aspergillus ustus	8.33
Aspergillus versicolor	8.33
Aspergillus terreus thom	8.33
Aspergillus flavus	8.33
Fusarium oxysporum	8.33
Fusarium solani	8.33

4. Conclusion

This survey underlined the microbiological quality and the risk of contamination of smoking fishes marketed in the lakeside village of Guezin by aflatoxigenic fungi. Microbiological standards have not been established for processed smoking fish in Benin. Due to the significance of the oral route transmission for many bacterial food-borne diseases, basic hygiene measures could assume a decisive importance in food safety management. It is also advisable that more attention (in the production, storage and selling methods) should be paid to its microbial quality and relevant quality control unit should be reactivated to assess the quality of read to eat foods also marketed in the lakeside village of Guezin.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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