ORIGINALE ARTICLE

IMPACT OF TRADITIONAL SMOKING AND SUN-DRYING ON THE MICROBIOLOGICAL QUALITY AND SAFETY OF CLARIAS GARIEPINUS AND OREOCHROMIS NILOTICUS HARVESTED FROM THE WHEDOS OF THE UPPER OUEME DELTA, BENIN

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ABSTRACT

Background : Fish is a crucial source of animal protein for consumers. In Benin, traditional processing methods, aimed at conservation, sometimes lead to contamination. **Objective** : The general aim of this study was to assess the microbiological quality of fresh, smoked, and smoked-dried Clarias gariepinus and Oreochromis niloticus fish caught in the "Whédos" of the communes of Aguégués, Adjohoun, Bonou, and Dangbo. **Methods** : A total of 256 samples of both fish species in fresh, smoked, and smoked-dried form were aseptically transported to the laboratory for various microbiological analyses. Microbial loads of hygiene and spoilage indicator organisms were enumerated using standard microbiological techniques. **Results** : Evaluation of the microbiological quality of the samples revealed that, overall, fresh fish (57%) were more contaminated than immediately hot-sampled smoked fish (9%), which were of satisfactory microbiological quality. Samples of smoked-dried fish (34%) were of unsatisfactory microbiological quality. It should be noted that the smoking technique used has a positive impact on the microbiological quality of smoked fish, but exposure to the sun at the end of the drying process, as practiced in the study area, is the main critical point which favors recontamination of the fish by the germs studied. Due to the heat-resistant nature of these germs, which secrete enterotoxins responsible for food poisoning, these smoked-dried fish present a problem for consumer health. **Conclusion** : The study highlights that smoked fish showed improved microbiological quality, whereas smoked-dried fish were of unsatisfactory quality due to recontamination. The traditional drying process, specifically exposure to the sun, poses a significant risk. Considering the heat-resistant nature of the bacteria involved, these findings underscore the health risk associated with consuming smoked-dried fish.
Keywords *: Clarias gariepinus, Oreochromis niloticus, Whédos, microbiological quality, Benin.*

1. INTRODUCTION

Fish and fish products in general are essential sources of nutrients to ensure a good nutritional and sanitary balance for both humans and livestock. Since 1961, annual global growth in fish consumption has been double that of population growth, demonstrating that the fishing sector is key to effectively combating hunger, malnutrition and poverty (Latifou *et al*., 2019). They contribute around 60% of the world's protein supply and over 30% of animal protein intake in developing countries (FAO, 2000). The growing scale of aquaculture production is leading to intensification and profound changes in traditional farming systems, particularly in South-East Asia and sub-Saharan Africa (Sèdogbo *et al*., 2019). The Republic of Benin has not been left out of this boom in aquaculture and fish farming in particular. According to data from INSAE (2013), total supply from the fisheries sector (fishing and aquaculture) stood at over 100,000 tonnes in 2013, compared with 93,700 tonnes in 2006 and 72,670 tonnes in 2003; an increase of almost 30% (Sèdogbo *et al*., 2019).

In view of the world's expanding population, the demand for fishery resources is constantly increasing. According to studies by Toko (2007), this is forcing the country to turn to imports of frozen fish, the volume of which has exceeded national production since 2005. It is therefore important not only to ensure greater availability of fishery products, but also to reduce the exploitation of natural fishery resources and the import of frozen products, which creates a dangerous country's dependence (Toko, 2007). Nevertheless, fishermen still carry out various types of traditional fish farming."*Whédos*", or fish holes, are another traditional form of fish farming devised by fishermen to take advantage of the succession of high and low water in flood plains (Sèdogbo *et al*., 2019). The species caught and reared here are mainly *Clarias gariepinus* and secondarily *Oreochromis niloticus* because of their ability to adapt to the water quality of these "*Whédos*" (Elegbe *et al*., 2015). However, this intensification of fish production remains critical to control, as the high density of farmed fish and low water renewal provide the conditions for the frequent occurrence of epizootics in fish farming (Sèdogbo *et al*., 2019). Despite the consequences amounting to hundreds of millions of US dollars each year (FAO, 2007), estimating the socio-economic and environmental impact of epizootics and enzootics in tropical fish farming is tricky. Moreover, fresh fish are highly perishable. To limit post-capture losses, various preservation methods such as frying, drying, salting and smoking have been developed. These preservation methods aim not only to prevent the proliferation of spoilage micro-organisms, but also to slow down certain biochemical reactions. Based on the above information, it was found that the artisanal nature of fish smoking and the lack of hygiene in production considerably increase microbial contamination of the products. The present study was therefore initiated with the general aim to assess microbiological quality of fresh, smoked and dried smoked fish from *Clarias gariepinus* and *Oreochromis niloticus*, two fish species caught in the "Whédos" of the upper Ouémé delta in Benin.

2. Materials and methods

2.1. Study framework

The geographical area covered by this study includes the four communes of the Ouémé valley in Benin, namely: Aguégués, Adjohoun, Bonou and Dangbo. The geographical positions of these different areas are presented on Figure 1.

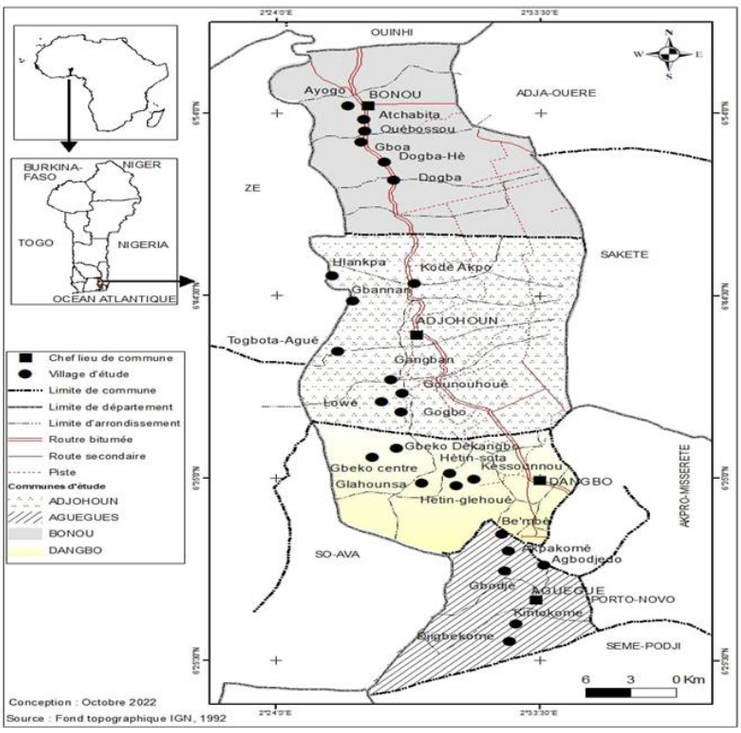

Figure 1 : Map of sampling area.

2.2. Smoking and drying technologies

The technological diagram used in this study for processing fresh *C.gariepinus* and *O.Niloticus* fish caught from "*Whédos*" in the communes of Aguégués, Adjohoun, Bonou and Dangbo is summarized in Figure 2.

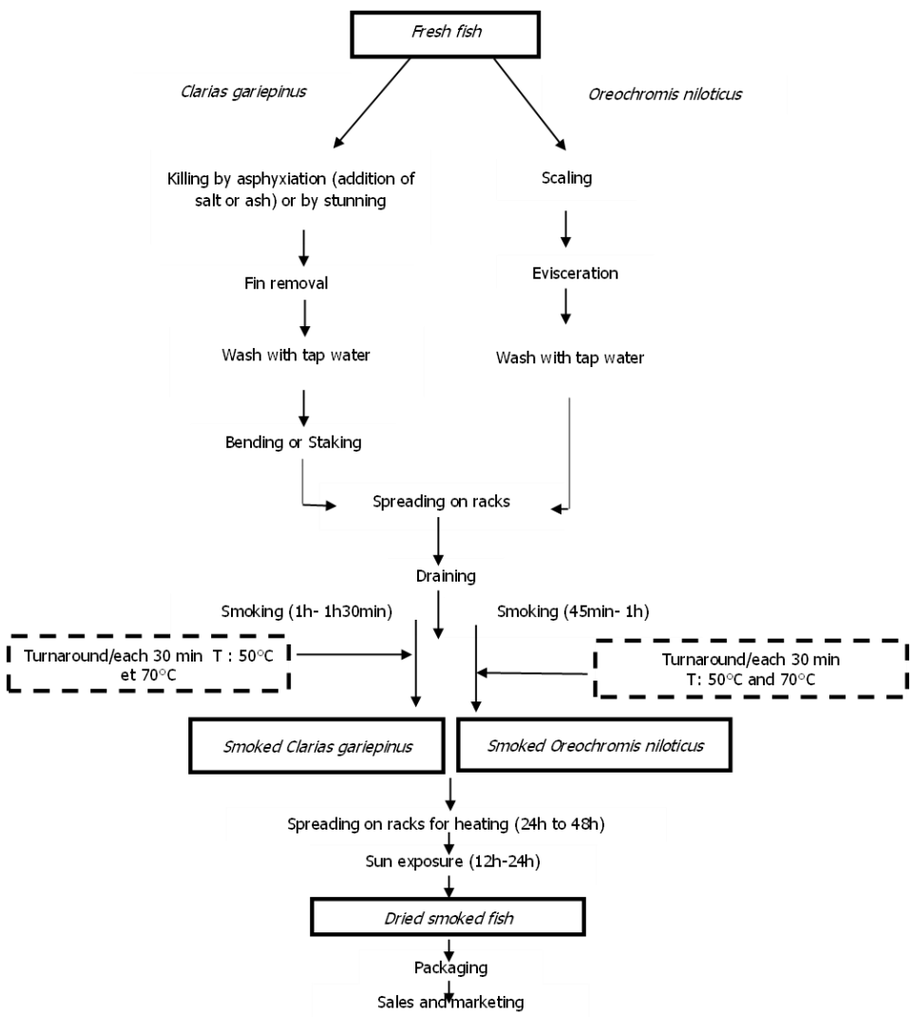

Figure 2 : Smoking and drying technology diagram for *Clarias gariepinus* and *Oreochromis niloticus.*

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2.3. Sampling

The biological material consisted of samples of fresh, smoked and smoked-dried fish of *C. gariepinus* (called *Asson* in the local Fon language) (Figure 3) and fresh, smoked and smoked-dried fish of *O. niloticus* (called *Akpavi* in the local Fon language) (Figure 4).

[A]: Fresh *Clarias gariepinus*; **[B]:**Smoked *Clarias gariepinus*; **[C]:**Smoke-dried *Clarias gariepinus*.
Figure 4: *Clarias gariepinus* (fresh, smoked and smoked-dried).

Samples of fresh, smoked and dried fish were collected under aseptic conditions to avoid further contamination. Sampling was carried out in each "*Whédos*" during flood periods. In each commune, at least four (4) villages were visited, and in each village at least eight (8) "*Whédos*" were sampled. In each "*Whédos*", each of the two (2) fish species was sampled at random. In total, two hundred and fifty-six (256) samples of the two fresh fish species were sampled. These fish were divided into three parts, of which one part (88) was kept fresh, another part (84) was processed into smoked fish and the remainder (84) was processed into smoked-dried fish. The fish were aseptically sampled in stomachers and transported to the laboratory in a 4°C cooler. Once at the laboratory, they were also stored in a refrigerator at 4°C, pending microbiological analysis. In the laboratory, the superficial and deep parts of the fish were aseptically harvested under a laminar flow hood, using sterile forceps.

2.4. Microbiological analysis methods for fresh, smoked and dried smoked fish

The microbiological germs investigated in the course of this work are essentially total aerobic mesophilic flora, yeasts and molds, total coliforms, fecal coliforms, *Staphylococcus aureus* and Enterobacteriaceae. The standards used are those of : CQIASA (2009); AFNOR (1996); AFNOR (2000); Jouve (1996) and Regulation 2073/2005 EC relating to microbiological criteria for fresh, smoked and dried fish. Microbial loads of hygiene and spoilage indicator organisms were enumerated using standard microbiological techniques described by Noumavo *et al*. (2023). Total aerobic mesophilic microorganisms were counted on Plate Count Agar (Oxoid, England) after incubation at 30°C for 72 h. Yeasts and molds were counted on Sabouraud agar (Biokar Diagnostics, France) supplemented with Chloramphenicol at 25°C for 5 days. Total coliforms and faecal coliforms were isolated on Violet Red Bile Lactose agar (Liofilchem Diagnostici, Italy) after incubation at 30 and 44°C, respectively, for 24 h. *Staphylococcus aureus* was isolated on Baird Parker agar (Biokar Diagnostics, France) supplemented with egg yolk and potassium tellurite after incubation at 37°C for 48 h. Finally, Enterobacteriaceae were counted on Methylene Blue Eosin agar (Biokar Diagnostics, France) at 37°C for 24 h.

2.5. Data processing and statistical analysis

The results of the microbial counts studied were entered using Microsoft Excel 2016 spreadsheet software. The same spreadsheet was used to calculate the microbial load in the fish and to express the results in the form of scientific graphs, enabling means to be compared between the data. The results were subjected to an Analysis of Variance (ANOVA) and Student-Newman-Keuls (SNK) test to compare the means between the microbial loads of contamination in the fish studied, and to determine significant differences between the means of the four study communes. The difference was considered statistically significant when the p-value < 0.05. The geographical map of the fish smoking, drying and sampling areas was produced using Qgis software version 2.14.

3. RESULTS

3.1. Microbiological quality of fresh fish samples

Figure 5 provides the results of microbial analyses conducted on fresh fish samples of both Clarias gariepinus and Oreochromis niloticus species, collected from four different communes (Aguégués, Adjohoun, Bonou and Dangbo). The

level of contamination is expressed as colony forming units per gram of sample (CFU/g). Higher CFU/g indicates greater microbial load and inferior microbiological quality. The data shows that across all communes, C. gariepinus generally exhibited higher microbial loads compared to O. niloticus. Within each species, there was also variation in contamination levels between communes. Specifically, C. gariepinus samples from Aguégués recorded the highest average microbial count of 4.1x102 CFU/g. Samples from Adjohoun and Bonou also surpassed the maximum acceptable limit of 1x102 CFU/g. Only C. gariepinus from Dangbo was within this limit. For O. niloticus, fish from Aguégués and Adjohoun had average counts higher than the limit, while those from Bonou and Dangbo were below. Overall, the results demonstrate differences in microbial quality between the two fish species and across sampling locations. C. gariepinus appeared more susceptible to contamination compared to O. niloticus. Fresh fish from Aguégués and Adjohoun communes generally showed inferior microbiological status.

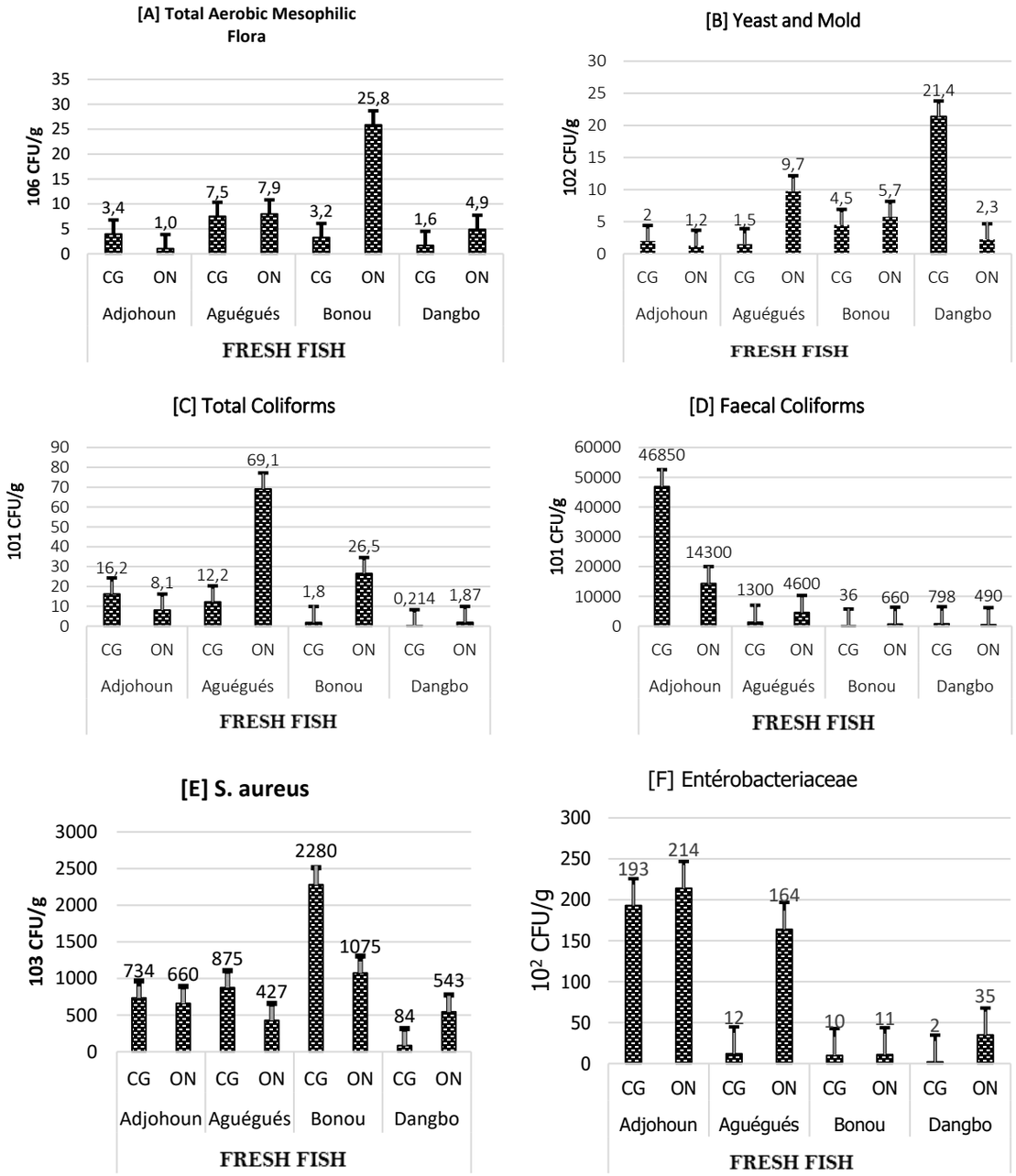


Figure 5: Level of contamination of fresh fish by hygiene and spoilage indicators.
***CFU/g**: Colony Forming Unit per gram of sample; **CG:** Clarias gariepinus; **ON**: Oreochromis niloticus.*

3.2. Microbiological profile of smoked fish samples

Figure 6 shows the results of microbial analyses conducted on smoked fish samples of Clarias gariepinus and Oreochromis niloticus species obtained from the four communes.

The contamination level is expressed as colony forming units per gram (CFU/g), with lower counts indicating better microbiological quality. For C. gariepinus, smoked samples from all communes had average CFU/g below the established limit of 1x102 CFU/g. Aguégués samples recorded the highest average count of 7 CFU/g. Regarding O. niloticus, smoked fish from Aguégués, Adjohoun and Bonou were within the limit, with averages below 10 CFU/g. Only samples from Dangbo showed a slightly elevated average of 1.3x102 CFU/g. Overall, smoked fish of both species presented lower microbial loads compared to fresh fish. C. gariepinus maintained better quality than O. niloticus after smoking. Additionally, smoked samples from Aguégués and Adjohoun- the communes with more contaminated fresh fish - exhibited the highest yet satisfactory CFU/g counts among the smoked groups. Overall, the results demonstrate that the smoking process improved the microbiological status of fish across the different sampling sources investigated. The reduction in microbial counts suggests smoking was effective in controlling hygiene and spoilage microorganisms.

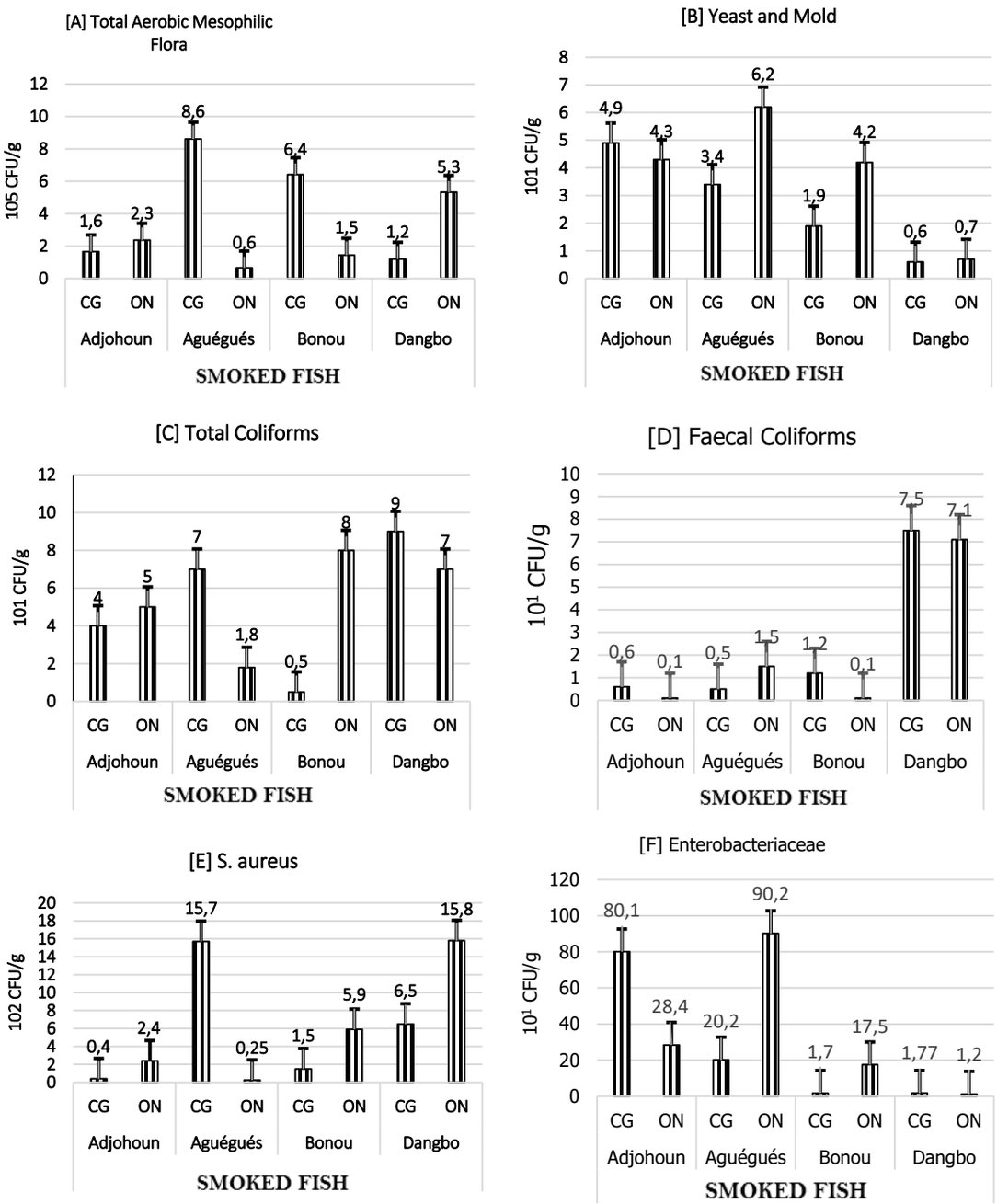
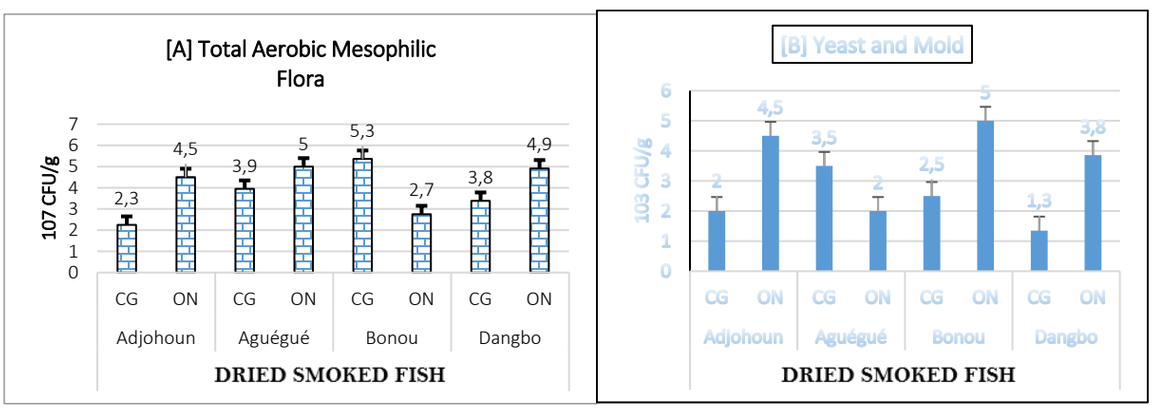


Figure 6: Level of contamination of smoked fish by hygiene and spoilage control germs.
***CFU**/g: Colony Forming Unit per gram of sample; **CG**: Clarias gariepinus; **ON**: Oreochromis niloticus*

3.3. Microbiological profile of smoked-dried fish samples

The overall level of contamination of smoked fish by hygiene and spoilage indicator germs, by species and by commune, is shown in Figure 7.



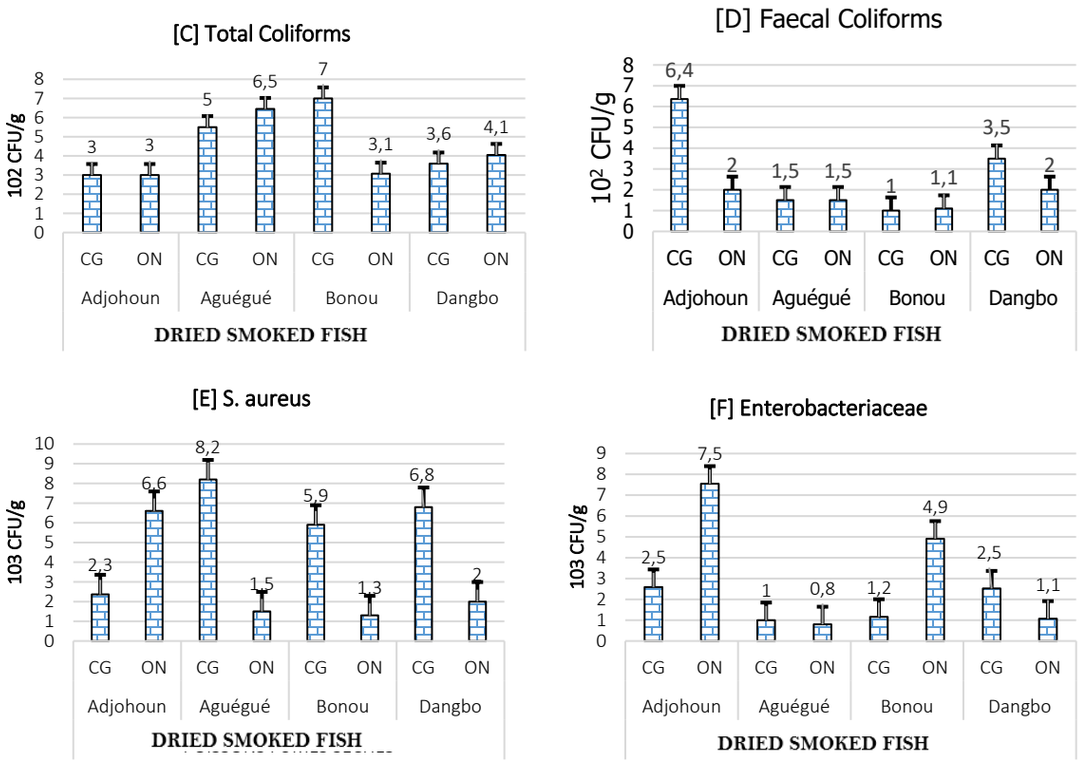


Figure 7: Level of contamination of dried smoked fish by hygiene and spoilage indicator germs.
***CFU**/g: Colony Forming Unit per gram of sample; **CG:** Clarias gariepinus/ ON= Oreochromis niloticus.*

4. DISCUSSION

Fish processing in the upper Ouémé delta is mainly carried out by non-literate women, using traditional procedures. This preservative processing of fish is sometimes a source of contamination of smoked and smoked-dried fish when good hygiene and production practices are not strictly observed during smoking and drying. Microbiological analysis of the samples collected for Total Aerobic Mesophilic Flora (TAMF) revealed that 65% of fresh fish samples from all origins had a fairly high average microbial load, above the threshold recommended by the Quebec standard (10^6 CFU/g) (CQIASA, 2009). The samples analyzed were heavily contaminated with germs indicative of general spoilage. These results can be explained by the failure to observe good hygiene practices around the "*Whédos*", the failure to clean fishing equipment and the fact that water in the "*Whédos*" remains the same for months without renewal, unlike that in lakes and rivers. Thus, our results are contrary to those of Brito *et al*. (2021) who observed a substandard microbial load on fresh local fish of *Clarias gariepinus* and *Oreochromis niloticus* sampled around lakes in southern Benin. They also contradict those of Itongwa *et al*. (2019), who showed that fresh fish samples of *Lates niloticus* and *Oreochromis niloticus*, sold in markets in the city of Bukavu, had satisfactory microbiological quality for total aerobic mesophilic flora.

The total and faecal coliform microbial loads of the various fresh fish samples analyzed are above the threshold limit recommended by the AFNOR standard, (2000). These results are in line with those of Degnon *et al*. (2012), who showed that samples of fresh fish caught and marketed at Cotonou's industrial fishing port had high levels of total and thermo-tolerant coliforms. There is concordance between the present results and those of Brito *et al*. (2021), who demonstrated that some fresh fish samples have microbial loads of total and faecal coliforms in excess of the standard established by EC n°853/2004 and n° 2073/2005.

As regards the enumeration of staphylococci, yeasts and moulds and enterobacteria, the results show that all the fresh fish samples analyzed had microbial loads above the standard : 10^3 CFU/g; 10^2 CFU/g and 10^2/25g respectively (Jouve 1996; AFNOR, 2000). These results are consistent with those of Itongwa *et al*. (2019), but contrary to the results of Degnon *et al*. (2012) and Mouokeu *et al*. (2018) for staphylococci and enterobacteria. In fact, the presence of these germs in samples from the "*Whédos*" not only testifies to poor hygienic practices in these "*Whédos*" environment, but can also be explained by the fact that fishermen sometimes suffer from infected skin lesions on their hands, or dermatophyte mycoses on their feet, which they may not have been able to protect properly during fish farming activities.

Relative to fresh fish, all samples of smoked fish immediately hot-sampled after smoking showed a satisfactory level of contamination with regard to FMAT, total and faecal coliforms, yeasts and moulds, *Staphylococcus aureus* and enterobacteria. These results are similar to those of Tossougbo (2017), who found that smoked fish generally had microbial loads in line with standards. It should be noted that the results obtained for *C. gariepinus* and *O. niloticus* smoked fish can be explained by the antibacterial activity linked to the smoking temperature, the germicidal power of the smoke or a synergy of action. During smoking, the smoke particles absorbed by the fish slow down bacterial growth on the product surface. The heat of the fire dries the fish, and if the temperature is high enough, the flesh cooks, thus preventing spoilage by micro-organisms and enzymes. However, the absence of these germs in the fish after pre-treatment and the smoking operation is partly an indication of the compliance with good hygiene practices by producers during fish smoking operations. Drying the fish flesh after smoking should also play a preservative role. With regard to the results obtained from sun-dried smoked fish, all samples are of unsatisfactory quality in terms of the germs studied. Our results are in good agreement with those of Degnon *et al*. (2012), Tossougbo (2017) and Atobla *et al*. (2022), who showed that exposure of smoked fish during

sale increases the risk of recontamination, generally from the sales environment, dust and the various manipulations of the finished product by saleswomen and customers during purchase. This recontamination generally leads to rapid spoilage of the product. The contamination of smoked fish exposed to the sun by certain germs, notably faecal coliforms, can constitute a public health problem, as these germs lead to the production of histamine, a heat-resistant biogenic amine that is toxic to humans (Huss, 2000). Thermotolerant coliforms are indicative of poor hygiene conditions, in this case staff hygiene, and are in fact hosts in the digestive tract of humans and animals, whose presence is generally due to contamination of fecal origin. The contamination of smoked fish exposed to sunlight by yeasts and moulds could be explained by the great capacity of these germs to grow on substrates with low water activity (Nguyen, 2007). Our results are in good agreement with those of Abdoullahi *et al*. (2019), who demonstrated the contamination of smoked and dried fish from Lake Fitri in the Batha region of Chad by fungal strains. In fact, smoked fish are left on smoking racks in the ambient air or kept in packaging for a long time before sale. This practice is thought to be the cause of post-contamination of smoked fish by fungi, despite low water activity (Jeantel *et al*., 2006). As for *S. aureus*, known to be common to the bacterial flora of human skin (Alabi *et al*., 2021), they were counted at a rate well above the norm in smoked-dried fish. However, these results are justified by the failure to observe good hygiene practices during smoking and especially during drying, as it is easy to see that the bare hand is widely used at all levels of the traditional fish processing process, starting with the raw material, which is fresh fish from the "*Whédos*", the workforce, the materials, the smoking and drying environment, and the succession of unit operations during processing. Added to this are excessive handling for sale by the sellers themselves (50%), the buyers (30%) and the action of vectors such as flies or insects. These observations, although not exhaustive, could explain the high load of certain germs such as *S. aureus* in smoked-dried fish. In fact, food poisoning due to *S. aureus* is not primarily the result of ingestion of the bacterium itself, but rather of toxins preformed in the contaminated food (Moloi *et al*., 2021). Staphylococcal food poisoning is mainly caused by a particular group of toxins called enterotoxins (Chebana *et al*., 2021). These enterotoxins are heat stable, highly toxic and persist in food even after adequate cooking (Fetsch *et al*., 2014; Ghalehnoo, 2018). In many cases, staphylococcal food poisoning (SSP) leads to a sudden onset of symptoms (1 to 6 hours after ingestion), with vomiting, abdominal pain and stomach cramps.

In view of the risks incurred by consumers of these fish, it is imperative to raise awareness and train both fish processors and resellers on the mandatory adoption of Good Hygiene Practices (GHP) and Good Production Practices (GPP) in their fish processing and marketing units to save the lives of consumers of this foodstuff highly prized by the Beninese population.

5. CONCLUSION

Traditional fish processing is subject to several contamination factors. The present study examined the effect of smoking and drying processes on the microbiological quality of *C. gariepinus* and *O. niloticus*, two fish species collected from the "*Whédos*" of the upper Ouémé delta in Benin. Fresh fish were found to be of mediocre microbiological quality, while smoked fish were of satisfactory microbiological quality. Smoked-dried fish, on the other hand, were of unsatisfactory microbiological quality. This contamination of the fish studied by hygiene and spoilage germs remains problematic. Improving traditional processing methods, particularly smoking and drying, would limit the proliferation of contaminants. The aim is also to raise awareness and train fishermen, processors and retailers in the mandatory adoption of Good Hygiene Practices (GHP) and Good Production Practices (GPP), in order to improve and guarantee the sanitary quality of fish processed in Benin's upper Ouémé delta.

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