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A Comparative Analysis of Microbial Load and Diversity in Fresh and Smoked Catfish (*Clarias gariepinus*) from Selected Markets in Delta State, Nigeria

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Abstract

This study evaluated the microbiological quality and safety of fresh and smoked catfish (*Clarias gariepinus*) obtained from five markets (Amai, Umutu, Abbey, Obiaruku, Kwale) in Delta State, Nigeria, during both rainy and dry seasons. A total of 50 samples were analyzed for total aerobic plate count, coliform count, and specific microbial isolates using standard microbiological and biochemical methods, and the results were benchmarked against Standards Organization of Nigeria (SON) guidelines. The findings showed that the microbial quality of fresh fish was variable, with gill samples from Amai and Obiaruku in the rainy season exhibiting marginal levels of contamination (10^3 CFU/g), whereas all smoked fish samples, irrespective of market or season, had satisfactory and significantly lower microbial loads (10^1–10^2 CFU/g), confirming the effectiveness of smoking as a preservation technique. Bacteriological analysis of fresh fish identified potential pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., and *Klebsiella* sp., while mycological assessment revealed the presence of *Aspergillus fumigatus*, *Aspergillus*

niger, *Mucor*, and *Fusarium* species. The study concludes that, although smoking markedly improves the microbiological safety of catfish, the initially high contamination levels in fresh fish, especially the occurrence of pathogenic bacteria and potentially mycotoxigenic fungi—pose a significant public health risk and indicate critical points of post-harvest contamination within the supply chain that require targeted control measures.

Keywords: *Clarias Gariepinus*; Microbial Load; Food Safety; Fish Smoking; Pathogenic Microorganisms.

INTRODUCTION

Fish constitutes a vital component of global food security, serving as a primary source of high-quality animal protein, essential long-chain omega-3 fatty acids, vitamins, and minerals for over three billion people worldwide. In Nigeria, as in many developing nations, the per capita consumption of fish is among the highest for animal proteins, driven by its relative affordability, cultural acceptance, and nutritional value. The African sharptooth catfish (*Clarias gariepinus*) is particularly significant in this context, dominating aquaculture and artisanal fisheries due to its resilience and high consumer demand (FAO, 2022).

However, the full nutritional benefits of fish are jeopardized by significant post-harvest losses and pervasive microbial contamination. Fish is an extremely perishable commodity due to its high moisture content, neutral pH, and presence of endogenous enzymes, making it a conducive medium for the rapid proliferation of microorganisms (Huss, 2018). In Nigeria and across West Africa, traditional smoke-drying is the most prevalent and economically viable preservation method. This process, which combines the antimicrobial effects of heat, dehydration, and phenolic compounds from smoke, is crucial for extending shelf-life, reducing losses, and imparting a desirable flavour (Sikorski & Kolakowski, 2010).

Despite its benefits, the safety of the final smoked product is intrinsically linked to two critical factors: the initial microbiological quality of the raw fish and the hygienic conditions maintained during handling, processing, and storage. Fresh fish can be contaminated from a multitude of sources, including polluted aquatic environments, unhygienic handling at landing sites, and unsanitary conditions in markets (Adebolu *et al.*,

2015). Indicator organisms, such as Total Aerobic Plate Count (TPC) and coliforms, provide a measure of the overall microbiological quality and hygiene. More critically, the presence of specific pathogens like *Staphylococcus aureus*, Escherichia coli, and *Salmonella* species poses direct and severe risks of foodborne intoxication and infection. The detection of *E. coli*, a fecal indicator, unequivocally points to contamination from unsanitary water or handling practices (Huss, 2018).

Furthermore, fungal contamination presents an insidious threat. Mycotoxigenic fungi, including Aspergillus and *Fusarium* species, can colonize fish products, particularly under poor storage conditions, and produce secondary metabolites known as mycotoxins. These toxins, such as aflatoxins from Aspergillus flavus and A. parasiticus, are stable, carcinogenic, and associated with a range of acute and chronic health effects, including immunosuppression and liver cancer (Ezekiel *et al*., 2019). Their potential presence in smoked fish indicates post-processing contamination, a point often overlooked in the supply chain.

While the Standards Organization of Nigeria (SON) has established microbiological guidelines to safeguard consumers, the enforcement and monitoring in traditional markets remain challenging. Existing studies on fish quality in Nigeria often focus either solely on fresh or smoked products, or are limited in their seasonal scope or microbial profiling. Therefore, a comprehensive, comparative, and seasonal assessment is critical. This study was designed to bridge this gap by systematically evaluating the microbiological load and diversity in both fresh and smoked *Clarias gariepinus* from five major markets in Delta State across different seasons. The specific objectives were to: (i) quantify and compare the TPC and coliform counts in fresh and smoked fish against SON standards; (ii) identify the specific bacterial and fungal species contaminating these products; and (iii) assess the seasonal variations in microbial quality, thereby providing a holistic view of the associated public health risks.

METHODS

Study Area

Ukwuani Local Government Area is found in Delta State, South – South Geopolitical Zone of Nigeria with the headquarters of the area in Obiaruku. The LGA is made up of several towns and villages which include Amai, Abbey, Kwale, Umutu and

Obiaruku. The estimated population of Ukwuani LGA is put at 212, 224 inhabitants with the area predominantly occupied by members of the Ndokwa Ethnic group. The Local Government is best known as the home of agriculture, oil and gas, and hospitality. Fishing also booms in Ukwuani LGA with the area's water bodies being rich in seafood.

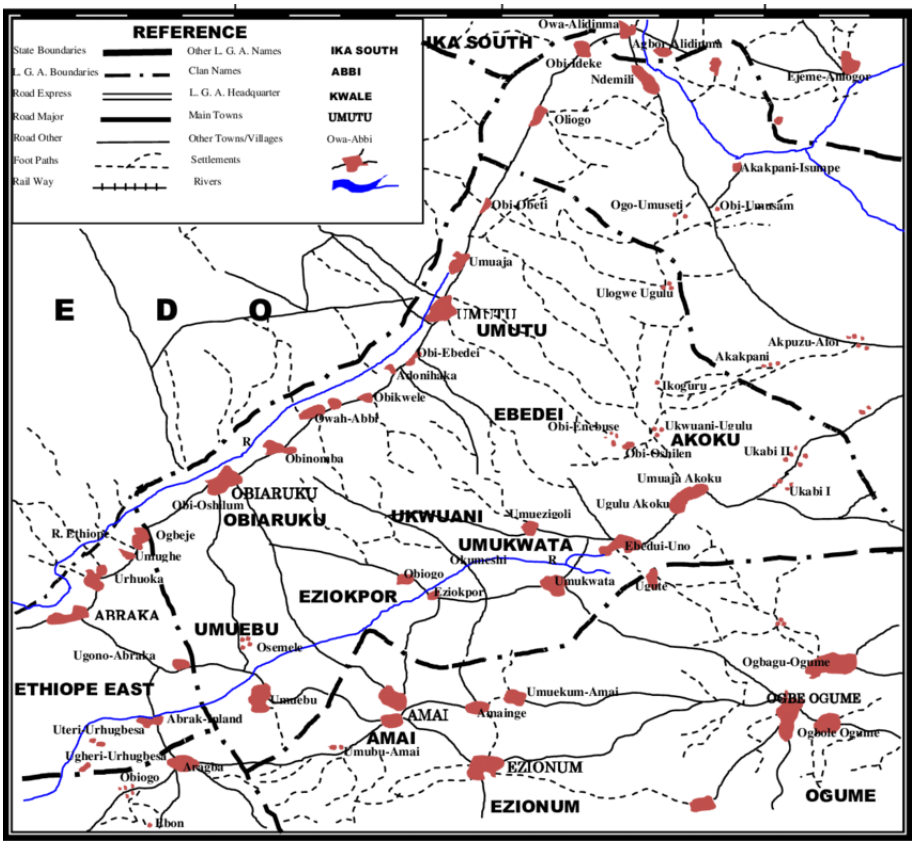

Figure 1: Map of Ukwuani Local Government Area. *Source*: Ukwuani Local Government Secretariat, 2022.

Sample Collection

A total of 50 (25 from each season and 5 from each community market) samples of smoked/dry and freshly caught Catfish (*Clarias gariepinus*) in wet and dry season from June to November, 2022 was randomly sourced from the fisher men/women as well as fish sellers at various markets in (Amai, Abbey, Kwale, Umutu and Obiaruku) Ukwuani communities. They were stored in plastic containers labelled and refrigerated prior to laboratory analysis.

Microbiological Analysis

In the laboratory, 10 grams of flesh and gill tissues from each fish were aseptically weighed and homogenized in 90 mL of sterile peptone water (0.1%) using a stomacher.

Serial dilutions were prepared, and 1 mL of appropriate dilutions was used for analysis. 1 mL of the homogenate was pour-plated using Plate Count Agar (PCA). Plates were incubated at 35°C for 48 hours, and colonies were counted as colony-forming units per gram (CFU/g). Distinct colonies from PCA plates were sub-cultured on Nutrient Agar. Pure isolates were identified based on Gram staining and a series of biochemical tests including catalase, coagulase, oxidase, indole, citrate, and urease, as per standard methods (Cheesbrough, 2006).

For mycological analysis, 1 mL of the homogenate was spread on Potato Dextrose Agar (PDA) acidified with lactic acid. Plates were incubated at 25°C for 3-7 days. Fungal isolates were identified based on macroscopic (colony morphology, colour, growth rate) and microscopic (hyphal and spore characteristics) features (Samson *et al.*, 2019).

Data Analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. The mean microbial counts were compared with the SON acceptable limit of 4 x 10^2 CFU/g for both TPC and coliforms. The SON classification was used: Satisfactory (<10^3 CFU/g), Marginal (<10^4 CFU/g), and Unsatisfactory (>10^5 CFU/g).

RESULTS

Table 1 below represent the Microbial loads of fresh fish samples analyzed from five markets during rainy season and standard control limit as recommended by standard organization of Nigeria (SON). The result showed that the microbial load from flesh and gills part of the fish samples analyzed from Abbey, Umutu, Kwale were satisfactory (10^2) except Amai and Obiaruku with marginal level (10^3) at the gills portion of the fish.

Table 2 below represent the Microbial loads of smoked fish samples analyzed from five markets during rainy season and standard control limit as recommended by standard organization of Nigeria (SON). The results showed that the microbial load from flesh and gills part of the fish samples analyzed from Amai, Abbey, Umutu, Kwale and Obiaruku were all satisfactory (10^2) at the flesh and gills portion of the fish.

Table 3 below represent the Microbial loads of smoked fish samples analyzed from five markets during dry season and standard control limit as recommended by standard organization of Nigeria (SON). The results showed that the microbial load from flesh and

gills part of the fish samples analyzed from Amai, Abbey, Umutu, Kwale and Obiaruku were all satisfactory (10^2) at the flesh and gills portion of the fish.

Table 4 below represent the total heterotrophic microbial count of catfish (*Clarias gariepinus*) obtained from five different markets and standard control limit as recommended by standard organization of Nigeria (SON). The results showed that the microbial (bacterial and fungal) load from flesh and gills part of the fish samples analyzed from Amai, Abbey, Umutu, Kwale and Obiaruku varies from satisfactory (10^2), marginal (10^4) and unsatisfactory (10^5) levels at the flesh and gills portion of the fish.

Table 5 below represent bacterial isolates and their biochemical Characteristics from fresh and dry fish (*Clarias gariepinus*) samples purchased from Amai, Abbey, Umutu, Kwale and Obiaruku

Table 6 below represent mycological isolates and their Characteristics in potato dextrose agar (PDA) from fresh and dry fish (*Clarias gariepinus*) samples purchased from Amai, Abbey, Umutu, Kwale and Obiaruku.

Table 1: Microbial loads of fresh fish samples from five markets during rainy season and standard control limit

Location	Parts isolated from	Total plate count (cfu/g)	Coliforms counts (cfu/mL)	(SON) $4x10^2$ cfu/mL (AL)
Amai	Flesh	1.7×10^3	1.7×10^3	Satisfactory
	Gills	1.2×10^2	1.0×10^2	Satisfactory
Abbey	Flesh	2.2×10^2	1.4×10^2	Satisfactory
	Gills	2.7×10^2	2.6×10^2	Satisfactory
Umutu	Flesh	$1.6X\ 10^2$	1.5×10^2	Satisfactory
	Gills	$2.3X\ 10^2$	2.0×10^2	Satisfactory
Kwale	Flesh	2.4×10^2	2.2×10^2	Satisfactory
	Gills	1.8×10^2	1.1×10^2	Satisfactory
Obiaruku	Flesh	2.3×10^2	2.1×10^2	Satisfactory
	Gills	2.0×10^3	2.0×10^3	Marginal

Data are means of triplicate determinations cfu/ml = colony forming unit per mill; AL = acceptable level, satisfactory $<10^3$; Marginal $<10^{4;}$ Unsatisfactory $>10^5$ SON = Standards Organization of Nigeria.

Table 2: Microbial loads of smoked fish samples from five markets and standard control limit during rainy season

Location	Parts isolated from	Total plate count (cfu/g)	Coliforms counts (cfu/mL)	(SON) $4x10^2$ cfu/mL (AL)
Amai	Flesh	1.0×10^1	1.0×10^1	satisfactory
	Gills	1.1×10^1	$1.01X\ 10^1$	satisfactory
Abbey	Flesh	$1.01X\ 10^1$	$1.01X\ 10^1$	satisfactory
	Gills	1.2×10^1	$1.2X\ 10^1$	satisfactory
Umutu	Flesh	$1.01X\ 10^1$	$1.01X\ 10^1$	satisfactory
	Gills	$1.01X\ 10^1$	$1.01X\ 10^1$	Satisfactory
Kwale	Flesh	$1.01X\ 10^1$	$1.01X\ 10^1$	Satisfactory
	Gills	$1.01X\ 10^1$	$1.01X\ 10^1$	Satisfactory
Obiaruku	Flesh	$1.01X\ 10^1$	$1.01X\ 10^1$	Satisfactory
	Gills	$1.01X\ 10^1$	$1.01X\ 10^1$	Satisfactory

Table 3: Microbial loads of Fresh Fish Samples from Five Markets and standard control limit during dry season

Location	Parts isolated from	Total plate count (cfu/g)	Coliforms counts (cfu/mL)	(SON) $4x10^2$ cfu/mL (AL)
Amai	Flesh	2.7×10^2	3.7×10^2	satisfactory
	Gills	3.8×10^2	3.0×10^1	satisfactory
Abbey	Flesh	4.7×10^2	2.4×10^2	satisfactory
	Gills	2.7×10^2	2.6×10^2	satisfactory
Umutu	Flesh	2.7×10^2	4.7×10^2	satisfactory
	Gills	2.7×10^2	3.2×10^2	Satisfactory
Kwale	Flesh	2.7×10^2	2.7×10^2	Satisfactory
	Gills	2.7×10^2	2.7×10^2	Satisfactory
Obiaruku	Flesh	2.7×10^2	2.7×10^2	Satisfactory
	Gills	2.7×10^2	2.7×10^2	Satisfactory

Table 4: Total Heterotrophic Microbial Count of Catfish (*Clarias gariepinus*) obtained from five different markets

S/N	Location	Parts isolated from	Bacteria counts	Fungal counts	(SON) AL
1	Amai	Flesh	5.4×10^4	1.3×10^2	Marginal
		Gills	4.0×10^5	1.5×10^1	satisfactory
2	Abbey	Flesh	1.6×10^3	2.0×10^3	Satisfactory
		Gills	7.2×10^3	1.0×10^2	Satisfactory
3	Umutu	Flesh	6.0×10^4	1.7×10^4	Marginal
		Gills	4.8×10^4	1.7×10^4	Marginal
4	Kwale	Flesh	$1.4X10^2$	1.2×10^2	Satisfactory
		Gills	2.1×10^1	1.8×10^2	Satisfactory
5	Obiaruku	Flesh	3.2×10^3	1.6×10^2	Satisfactory
		Gills	1.3×10^2	1.1×10^2	Satisfactory

Data are means of triplicate determinations cfu/ml = colony forming unit per mill; AL = acceptable level, satisfactory $<10^3$; Marginal $<10^{4;}$

Unsatisfactory $>10^5$ SON = Standards Organization of Nigeria.

Table 5: Bacterial isolates and their biochemical Characteristics

S/No	Isolates	Urease	Indole	Citrate	Oxidase	Gram Reaction	Coagulase
1.	*S. aureus*	+	-	+	-	+	+
2.	*E. coli*	-	+	-	-	-	-
3.	*Salmonella* sp.	-	-	-	-	-	-
4	*Klebsiella* sp.	+	-	+	-	-	-
5	*Proteus* sp.	+	-	+	-	-	-
6	*Streptococcus* sp.	+	-	-	-	+	-

Table 6: Mycological analysis using potato dextrose agar (PDA)

Locations	Possible isolates	Growth rate		
Amai	Mucor	3-4 days (Slow)	Yellow-white fluffy with brown reverse sides	Hyphae without rhizoids, large globose sporaniphore

Locations	Possible isolates	Growth rate		
Abbey	*Aspergillus fumigatus*	2-5 days (Slow)	Creamy yellow filamentous colonies	Large/globose conidiphore, loose columna with biserited hypha
Umutu	*Fusarium* spp.	2-3 days (Fast)	Whitish cotton aerial	Enlongated ovoid curved microconidia
Obiaruku	*Aspergillus niger*		White to yellow but later turns distinct black as colony develops	Large conidiosphore with 2 series of sterigmata over its entire surface, Brown to black conidia and rough walled spores, black and green
Kwale	Yeast	3-5 days (Slow)	Creamy white	B-polar budding cells with lemon- shaped mother tips

DISCUSSION

This study provides a comprehensive and comparative analysis of the microbiological landscape of catfish in Delta State, revealing critical insights into contamination sources, the efficacy of traditional processing, and persistent public health risks. The analysis of fresh fish revealed a clear pattern of contamination, with gill samples consistently showing higher microbial loads than flesh samples. This is anatomically expected, as gills function as a filtration organ, directly interacting with the aquatic environment and trapping suspended microorganisms (Adebolu *et al*., 2015). The marginal microbial levels (10^3 CFU/g) found in the gills of fresh fish from Amai and Obiaruku during the rainy season are particularly telling. This seasonal spike can be directly attributed to increased run-off from agricultural and urban areas during heavy rains, which introduces a higher load of nutrients and microorganisms into aquatic ecosystems. This finding aligns with studies by Gyimah *et al.* (2020), who reported significantly higher bacterial counts in fish during the wet season in Ghanaian coastal waters, linking it to land-based pollution wash-off.

The most pronounced finding of this study is the dramatic and consistent reduction in microbial load in all smoked fish samples. The TPC and coliform counts plummeted to remarkably low levels (10^1 CFU/g), which are not only "satisfactory" but indicative of a highly effective preservation process. This result robustly demonstrates the combined efficacy of the thermal and desiccative phases of smoking. The heat from smoking lethally affects mesophilic bacteria, while the subsequent reduction in water activity creates a

hostile environment that prevents the germination of surviving spores and the growth of any post-process contaminants (Sikorski & Kolakowski, 2010). This transformative effect of smoking on microbial quality has been consistently documented in similar contexts, such as in the work of Akinola *et al.* (2016) on smoked fish in Southwestern Nigeria.

Despite the efficacy of smoking, the isolation of specific pathogenic bacteria from the fresh fish samples raises substantial public health concerns. The presence of *Staphylococcus aureus*, identified by its Gram-positive reaction and positive coagulase test, is a strong indicator of human contamination, likely originating from the hands, nasal passages, or skin of fish handlers (Cheesbrough, 2006). This pathogen is a leading cause of food intoxication due to its heat-stable enterotoxins. More alarmingly, the isolation of *E. coli* and *Salmonella* sp. serves as an unequivocal marker of fecal contamination. This suggests that the fish were harvested from or processed with water contaminated with sewage or animal waste. The persistence of these pathogens, even in low numbers post-smoking, is a significant risk. If the smoking process is inadequately controlled (e.g., insufficient temperature or time), or if cross-contamination occurs after processing, these pathogens can proliferate to infectious doses. This finding is consistent with reports from other regions, where similar pathogens have been isolated from smoked fish, highlighting a systemic issue in artisanal fish processing across West Africa (Sofolahan *et al.*, 2017).

The mycological analysis unveiled a different dimension of risk. The isolation of species such as *Aspergillus fumigatus*, *A. niger*, and *Fusarium* spp. is particularly concerning. Unlike bacteria, which are primarily controlled by the smoking heat, fungal spores are highly resistant and can easily re-contaminate the product during cooling, packaging, or storage in humid, dusty environments. Aspergillus niger and related species are known potential producers of ochratoxins and fumonisins, while certain *Fusarium* species produce trichothecenes and fumonisins (Ezekiel *et al.*, 2019). The presence of these mycotoxigenic fungi implies a risk of mycotoxin accumulation, which is not eliminated by cooking and poses chronic health risks, including carcinogenic, teratogenic, and immunosuppressive effects. This points to a critical post-processing control point that is frequently neglected in traditional settings. However, the high initial load of pathogens and the subsequent fungal contamination indicate major gaps in hygiene at the beginning and end of the supply chain. This comparative analysis underscores that interventions focusing solely on the smoking process are insufficient. A more effective strategy, as suggested by this study, would be an integrated approach that combines public education on hygienic handling of fresh fish,

improvement of market and storage sanitation, and the promotion of simple, low-cost protective packaging for smoked products to prevent fungal re-contamination.

CONCLUSION

This study demonstrates that while traditional smoking is a highly effective method for reducing the microbial load in catfish to safe levels, the initial contamination of fresh fish with potential pathogens and toxigenic fungi remains a significant challenge. The presence of *E. coli*, *Salmonella*, and Aspergillus species indicates breaches in hygiene from the point of harvest to the market. Therefore, public health interventions must focus not only on the final product but also on the entire supply chain. Recommendations include:

1. Educating fishers and traders on improved hygienic handling practices.

2. Ensuring the use of clean water sources for fishing and initial processing.

3. Implementing proper storage conditions for smoked fish to prevent post-processing fungal contamination.

4. Strengthening regulatory monitoring and enforcement of food safety standards in local markets.

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