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Microbial Quality of traditionally smoked *Clarias gariepinus* and *Micromesistus poutassou* in local markets in River state, Nigeria

Lukman Adegoke Agbabiaka[1,2*], Olatunji Abubakar Jimoh[3] and Gerald Ikenna Iwuagwu[2]

[1]Department of Fisheries Technology, Federal Polytechnic Nekede, Imo State, Nigeria. [2]Department of Fisheries Technology, Federal Polytechnic, Ekowe, Bayelsa State. [3]Agricultural Technology Department, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria *Author for correspondence. E-mail: agbabiakala@gmail.com

ABSTRACT. The present study evaluated microorganisms associated with traditionally smoked *Clarias gariepinus* and *Micromesistus poutassou* collected from three local markets (Mile One, Choba and Igwuruta) in Port Harcourt, River State, Nigeria. Bacterial and fungal analyses of the samples were carried out in the laboratory following standard procedures. The results showed wide variations in bacterial and fungal counts in smoked samples of *C. gariepinus* and *M. poutassou* collected from the three markets. Seven bacterial species (*Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus, Enterobacter sp, Escherichia coli, Salmonella sp*,) were identified in the samples. In smoked catfish, total bacterial counts ($4.2x10^5$ cfu) were significantly ($p < 0.05$) higher in samples from Mile One Market while the least counts ($1.64x10^5$ cfu) was observed in samples from Igwuruta Market. Samples from Choba market recorded significantly higher coliform and Salmonella counts when compared with the values recovered from the other two markets. Similarly, *E. coli* growth was observed only in samples from Choba market at $3.90x10^3$cfu. Based on public health concerns, the microorganisms identified in this study are causative agents of foodborne illnesses, hence the need to exercise caution in direct consumption of the fishes.

Keywords: contaminant; smoked fish; microbial counts; public health.

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Introduction

Fish has become an increasingly important food component for many people around the world, as it constitutes the essential source of quality animal protein and other elements necessary for proper functioning and maintenance of human body (Pal et al., 2015). In Nigeria and other developing countries, fish consumption goes beyond socio-economic, age, religious and educational barriers (Emikpe et al., 2011), due to its high-quality protein, other essential nutrients and omega 3-fatty acids, and its low cholesterol content as compared to other meats (Zanna et al., 2020). However, fish is a highly perishable commodity which must be preserved adequately to prevent spoilage and extend its shelf-life and usefulness after capture. Smoking is one of the preferable methods of preserving fish in many developing countries (Belichovska et al., 2019). This preservation technique gives the special colour and flavour to the fish as well as reducing water and inhibiting bacterial and enzymatic actions through the application of heat (Assogba et al., 2022). In major fishing communities, large proportions of fish are processed using traditional smoking kilns made of clay, cement blocks, drums or iron sheets.

Fish products processed from traditional smoking kilns are readily consumed by people of all categories, but there are reports indicating that these products may carry pathogenic bacteria and parasites of high potential risks to human health (Amoah et al., 2024). Furthermore, smoked fish products are usually hawked in hazardous manner by spreading them in a tray with no cover or on a table in open markets, while the fish can be touched with bare hands during pricing. These traditional marketing activities take no cognizance of risks of microbial contamination from the handlers (infected persons or carriers of pathogens) and the environment (contact surfaces, processing equipments and facilities) (Akinwumi & Adegbehinde, 2015). Aladejana et al. (2023) reported that smoked blue whiting fish from Nigeria showed high bacterial counts, with *E. coli* being the most prevalent at 45.46%. In a study conducted by Daramola et al. (2020) on smoked

Clarias gariepinus in Ota market showed that *Vibrio cholerae*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella spp* and *Salmonella typhi* were the bacteria isolated

Maintaining fish of high quality standard is a major concern to both the fish consumers and public health authorities in recent years, as fishery products are increasingly being associated with food safety issues (Vergis et al., 2021). Previous researchers have reported that microbial contaminations in fish are mostly due to improper storage and handling practice (Samira et al., 2024). Microbial contaminated fish products are reportedly involved in the transmission of pathogenic microorganisms and toxins to humans (Novoslavskij et al., 2016). According to Pal et al. (2015), the greatest risk to human health occurs due to the consumption of raw, inadequately cooked or insufficiently processed fish and fish products, thus pinpointing the need to monitor potential contaminants in fish products during production, processing and distribution activities to prevent unsafe foods reaching the consumers. This study therefore aimed at evaluating microbiological quality of smoked *Micromesistus poutassou,* (blue whiting) and *Clarias gariepinus* (African catfish) available for sale in local markets in River State, Nigeria

Material and methods

Sampling location and procedures

Smoked samples of *Micromesistus poutassou,* (blue whiting) and *Clarias gariepinus* (African catfish) used in this study were procured from three local markets, namely Mile one, Choba and Igwuruta markets in Port Harcourt, Rivers State, Nigeria. Three traders were randomly selected in each market, while two fish samples were collected from each trader. The samples were randomly selected from the bulk of smoked fish displayed for sale and were collected in sterile polythene materials, labelled and transported within thirty minutes of collection in cooling container to the laboratory for microbial analysis.

Experimental conditions

Each sample was crushed to fine particles in a mortar under aseptic conditions. 10 grams of each crushed sample was weighed using sterile filter paper on a sensitive weighing balance and aseptically poured into a 90 mL of sterile distilled water and thoroughly mixed. Then, 1 mL of the homogenate was taken and serially diluted four fold, after which 1 mL was inoculated into petri-dishes containing appropriately solidified culture media and spread with a sterile bent glass rod. Nutrient Agar, (NA), MacConkey Agar (MA), Eosin Methylene Blue Agar (EMBA), Salmonella-Shigella Agar (SSA) and Potato Dextrose Agar (PDA) were employed for the isolation/enumeration of total bacterial counts, coliform counts, *Eiccherichia coli* counts, *Salmonella* counts and fungal counts, respectively. The media were prepared according to the manufacturer's instructions.

The inoculated plates were incubated for 48 and 72 hours at temperature of 30°C for bacterial and fungi growths respectively. After incubation, plates having 30 to 300 colonies were enumerated and recorded as colony forming units (cfu), while their morphological characteristics were observed. Representative colony based on similar morphological characters from the culture plates were further subjected to biochemical tests, such as gram staining, catalase, motility, coagulase, citrate, oxidase, methyl red and Voges Prosceur and sugar fermentation test, as described earlier by Ekundayo et al. (2014).

Statistical analysis

The counts for bacteria and fungi recovered from the two fish species from the three markets were analyzed using One-way analysis of variance (ANOVA) and, where significant difference ($p < 0.05$) existed among means, they were separated using Duncan multiple range test. The data were log-transformed before being analyzed and the results were reconverted after analysis. The analyses were done in computer software SPSS package version 20.0. The values for both bacterial and fungal isolates were expressed as means and standard deviation.

Results

The results of microbial counts of smoked catfish and blue whiting collected in three local markets in Port Harcourt, River State, Nigeria, were presented in Tables 1 and 2. Wide variations in both bacterial and fungal counts were observed in the analyzed samples from the three markets. For the smoked catfish, total

bacterial counts (TBC) of $4.2x10^5$ cfu were significantly ($p < 0.05$) higher in samples from Mile One Market while the least values of $1.64x10^5$ cfu was observed in samples from Igwuruta Market. Higher values of coliform counts of $2.83x10^3$ cfu were observed in samples from Igwuruta Market but the count was not significantly different among the three markets. Growth of Salmonella/Shigella was not observed in samples from any of the markets, while *E. coli* count was recorded only in samples from Choba Markets. Significantly highest counts of fungi were recorded in samples from Mile One Marker, with the least values recorded in samples from Choba Market.

In smoked blue whiting, samples from Igwuruta and Mile One markets had significantly higher bacterial counts that those from Choba Market. Samples from Choba market recorded significantly higher coliform and Salmonella counts when compared with the values recovered from the other two markets. Similarly, *E. coli* growth was observed only in samples from Choba market at $3.90x10^3$ while growth was not observed in samples from Mile One and Igwuruta Markets. The highest fungal counts were observed in samples from Mile One Market at $6.0x10^5$ while the least values were observed in samples from Igwuruta Market.

The morphological and biochemical characteristics of the bacteria isolates recovered from the samples from the three studied markets were presented in Table 3. In all, seven bacterial species comprising of *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus, Enterobacter sp, Escherichia coli, Salmonella sp*, were identified. Similarly, the morphological characteristics of fungi isolates from all samples were presented in Table 4, where five fungi species, namely *Penicillium notatum, Aspergillus niger, Saccharomyces sp, Rhizopus nigricans* and *Fusarium sp.* were identified

Table 5 showed the distribution of bacterial and fungal isolates recovered from the different fish sampling markets. In smoked blue whiting, samples from Igwuruta Market had highest diversity of six bacterial species while Mile One Markets had least bacterial diversity of four. Similarly, the highest and lowest fungi diversities were from samples collected from Igwuruta Market and Mile One Markets respectively. For smoked catfish, samples from Igwuruta and Choba Markets had highest and the least bacterial diversities. Similarly, fish samples from Igwuruta and Choba had highest and the least fungi diversities respectively

Table 1. Counts of bacterial and fungal isolates of smoked *Clarias gariepinus* collected from three local markets in Port Harcourt, River State, Nigeria.

Microbial count	Mile One Market	Choba Market	Igwuruta Market
Total bacterial counts (cfu g^{-1})	$4.20±0.96×10^{5a}$	$3.30±1.05×10^{5b}$	$1.64±0.50×10^{5c}$
Coliform counts (cfu g^{-1})	$1.21±0.25×10^{3b}$	$2.21±0.93×10^{3a.b}$	$3.82±0.96×10^{3a}$
Salmonella/Shigella counts (cfu g^{-1})	BDL	BDL	BDL
E. coli (cfu g^{-1})	BDL	$3.10±0.59×10^{3a}$	BDL
Fungal counts(cfu g^{-1})	$9.00±2.11×10^{4a}$	$1.55±0.49×10^{4c}$	$5.20±1.65×10^{4b}$

Values with different superscripts across the same row are significantly different at $p < 0.05$. BDL – below detection limit.

Table 2. Counts of bacterial and fungal isolates of smoked *Micromesistus potassou* collected from three local markets in Port Harcourt, River State, Nigeria.

Microbial counts	Mile One Market	Choba Market	Igwuruta Market
Total bacterial counts (cfu g^{-1})	$2.49±0.36×10^{5a}$	$1.62±0.09×10^{5b}$	$5.50±1.55×10^{5a}$
Coliform counts (cfu g^{-1})	$2.83±0.71×10^{3a}$	$2.96±×10^{3a}$	$1.11±0.32×10^{3b}$
Salmonella/Shigella counts (cfu g^{-1})	BDL	$2.60±0.50×10^{3a}$	$1.09±0.07×10^{3a}$
E. coli (cfu g^{-1})	BDL	$3.90±1.35×10^{4}$	BDL
Fungal counts(cfu g^{-1})	$6.30±1.44×10^{5a}$	$2.16±0.82×10^{5b}$	$2.00±0.83×10^{5b}$

Values with different superscripts across the same row are significantly different at $p < 0.05$. BDL – below detection limit.

Table 3. Morphological and biochemical characteristics of bacterial species recovered from smoked fish samples obtained from the three studied markets in Port Harcourt, Nigeria.

Characteristics	Observation on Isolated bacteria						
	A	B	C	D	E	F	G
Colony shape and appearance	Smooth, moist and shiny, low convex, golden yellow colony on NA	Dull and dry serrated flat cream colony on NA	Black, fish-eye like colony on SSA but creamy on NA and MCA	Small, circular moist and shiny low convex, cream colony on NA	Circular, light pink colonies on MCA	Circular, convex, smooth, creamy colony on NA and MCA but glistering EMBA	Small circular low convex moist and shiny yellow colonies on NA

Gram reaction		Gram +	Gram +	Gram -	Gram +	Gram -	Gram -	Gram +
Cell Morphology		cocci mainly in clusters, few in parts	Large rods in short chains with central spores	rods in chains	cocci in long chains, few in pairs and clusters	rods in short chains	Short rods	Cocci predominantly in tetrads
Motility		+	-	+	+	-	-	-
Oxidase		-	-	+	-	-	-	-
Catalase		+	+	-	-	+	+	+
Coagulase		+	-	+	-	-	-	-
Indole		-	-	-	-	-	+	-
Methyl-Red		-	-	+	+	+	-	+
Voges-Proskaur		+	+	-	-	-	+	-
Spore forming		+	-	-	+	-	+	-
Fermentation of	Lactose	+	-	-	+	+	+	-
	Glucose	+	-	+	+	+	+	+
Most Probable Identity		*Staphylococcus aureus*	*Bacillus cereus*	*Salmonella sp*	*Enterococcus sp*	*Enterobacter sp*	*E. coli*	*Micrococcus sp*

NA – nutrient agar; MCA – Mac Conkey agar; SSA – salmonella shigella agar; EMBA – eosine methylene blue agar; "-" – negative; "+" – positive

Table 4. Macroscopic and microscopic characteristics of fungi isolated of smoked fish samples collected from open markets in Port Harcourt, Nigeria.

Isolate	A	B	C	D	E
Colony shape and appearance	Light brown to black powdery spores	Tall, white, filamentous hyphae, bearing black spores at its tips	Green spores enclosed in white mycelia	Short, whitish, cotton wool like, filamentous hyphae red at the reverse	Circular cream colonies
Microscopic morphology	Conidia globosed, vesicles attached to sterigmata.	Non septate hyphae which are irregular in size	Septate hyphae with mob-like conidia	Conidia arranged like comma or sickle	Large gram-positive spherical budding cells
Identity of Isolates	*Aspergillus niger*	*Rhizopus nigricans*	*Penicillium notatum*	*Fusarium sp*	*Saccharomyces sp*

Table 5. Distribution of bacteria and fungi from samples obtained from the three studied markets in Port Harcourt, Nigeria.

Microbial type	*Micromesistus potassou*			*Clarias gariepinus*		
	Mile One Market	Choba Market	Igwuruta Market	Mile One Market	Choba Market	Igwuruta Market
Bacterial isolates	*B. cereus, S. aureus, En. faecalis, Enterobacter sp*	*B. cereus, S aureus, E. coli, Enterobacter sp, M. luteus, Salmonella sp,*	*B. cereus, S. aureus, En. faecalis, Enterobacter sp, M. luteus, Salmonella sp,*	*B. cereus, S. aureus, En. faecalis,*	*E. coli, Enterobacter sp, S. aureus, B. cereus, En faecalis*	*B. cereus, S aureus, En faecalis, Enterobacter sp, ,*
Fungal isolates	*S cerevisiae, A. niger, P. notatum*	*R. nigricans, A. niger, S cerevisiae*	*A. niger, R. nigricans, P. notatum, S. cerevisiae*	*P. notatum, S. cerevisiae*	*Fusarium sp*	*A. niger, P. notatum, R. nigricans*

Discussion

The results obtained from this study showed that samples of both smoked catfish and blue whiting obtained from the three studied markets were highly contaminated with potentially harmful microorganisms. Total bacterial counts were observed to be slightly above the safety limit of 10^5 cfu g^{-1} recommended by International Commission on Microbiological Specif International Commission on Microbiological Specifications for Foods (ICMSF, 2000), while fungal counts were in the range of 10^4 – 10^5 cfu g^{-1} in the three studied markets. These findings are in line with several authors who reported similar number of contaminated microorganisms, including Esther et al. (2010) for street-vended and open marketed smoked blue whiting (*Micromesistus poutassou*); Nyarko et al. (2011) for smoked sardine (*Sardinella aurita*) obtained from market centre of Tema in Ghana; Yusuf and Hamid, (2012) for smoked *Clarias gariepinus* retailed in open markets in Bauchi, Nigeria; Adegunwa et al. (2013) for smoked herring (*Sardinella eba*) in Ogun State, Nigeria, and Ibrahim et al. (2014) in smoked *Clarias gariepinus* in Minna, Nigeria.

There were wide variations in both bacterial and fungal counts among the samples from the three markets. This variation could be due to differences in handling, processing, storage conditions, and hygiene

practices at the markets. Previous reports have shown that microbial contaminations of smoked fish usually occur due to unhygienic handling and exposure of the smoked fish products during processing, storage, distribution and marketing. According to Sabuj et al. (2018) and Birgen et al. (2020), the presence of flies and pests, dirty storing places, vending environments littered with garbage, filthy clothes, and lack of hygienic knowledge, are responsible for microbial contamination of ready-to-eat foods, including smoked fish. Similarly, fungal contamination may occur during hawking where the fish is exposed to fungal and fungal spores contamination from the environment. In the present study, smoked fish samples were observed to be displayed in open trays, exposing them to contact by insects, dust particles and even consumers who were observed to be touching the commodity with bare hands while haggling over prices. For smoked catfish, the total bacterial counts were significantly higher in samples from Mile One Market compared to Igwuruta Market. Similarly, for smoked blue whiting, samples from Igwuruta and Mile One markets had significantly higher bacterial counts than those from Choba Market. These differences could be attributed to the storage and processing methods employed at each market. Dike-Ndudim et al. (2014) further reported that smoked fish tend to reabsorb moisture when exposed to high humid conditions during storage and distribution which enhances the growth of the contaminant microorganisms that are inside and on the surface of the products.

Based on public health concerns, some of the microorganisms identified in the present study are known to be causative agents in some foodborne illnesses. For example, the presence of *Staphylococcus aureus* – a normal flora of skin and mucous membrane of humans – is often attributed to unhygienic human handling during processing and distribution of food (Taylor & Unakal, 2023). This bacterium is resistant to heat, drying and radiation, and is capable of producing toxins that cause gastroenteritis (Cheung et al., 2021) *Escherichia coli* has received more attention among all foodborne pathogens as it is a facultative anaerobic bacterium and is considered the most commonly encountered pathogen in the *Enterobacteriaceae* family. *E. coli* acts as a vital food hygiene indicator since its presence reflects the possibility of contamination by other enteric pathogens (Rahman et al., 2017), and, according to Navab-Daneshmand et al. (2018), the presence of *E. coli* in any food indicates possible feacal contamination. Odu and Imaku, (2013) enumerated diseases likely caused by *E. coli* to include diarrheal illness, urinary tract infections, meningitis, wound infections and dysentery.

Furthermore, the fungi of the genera *Aspergillus* and *Penicillium* isolated in this study are normal flora of environmental origin, but could produce spores that can survive even under harsh conditions. More so, these fungi have been linked with the production of different types of toxins (such as aflatoxin, ochratoxin and citrinin) of varying levels under diverse environmental conditions. The potency of these toxins appears not to be affected by cooking and may cause severe or fatal damage to the liver and kidney (Nazareth et al., 2014). It has also been reported that some fungi, such as *A. niger*, are allergenic (Martínez et al., 2021). Although smoked fish are generally classified as ready-to-eat food, the presence of these microbial contaminants isolated in this study emphasizes the need to exercise caution in direct consumption of smoked fish products to avoid foodborne diseases and other associated risks. This study highlights the importance of proper handling, processing, and storage practices in ensuring the microbial safety of smoked fish products. The presence of coliforms and *E. coli* in some samples indicates the need for improved hygiene practices at certain markets. However, the absence of Salmonella and Shigella is encouraging. Overall, these results can inform interventions to improve the safety and quality of smoked fish products in the region.

Conclusion

The present study isolated seven bacterial species (*B. cereus, S. aureus, E. faecalis, M. luteus, Enterobacter sp, E. coli,* and *Salmonella sp*) and five fungi species (*P. notatum, A. niger, Saccharomyces sp, R. nigricans* and *Fusarium sp.*) from smoked *M. poutassou,* (blue whiting) and *C. gariepinus* (African catfish) collected from local markets in River State, Nigeria. Some of these microorganisms are known to cause foodborne illnesses with serious fatality. Proper hygiene and sanitation conditions must therefore be maintained during the preparation, handling, and delivery of such foods to avoid contamination with foodborne pathogens

Ethical statement

The entire procedure complied with the guidelines for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the institution's Animal Research Ethics Committee. Ethics Reference No: FPE/FST/2023/34.

Data availability

Data will be made available on request.

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