

## Comparative Evaluation of Microbiological Quality and Public Health Risks of Fresh and Smoked Fish Sold in Selected Markets, Port Harcourt

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### Abstract

The microbiological quality of fresh and smoked fishes sold around selected markets (Mile 3, Mile 1 and Creek Road) in Port Harcourt City was carried out. The microbial count of the fresh fish was higher than that of the smoked ones. The bacterial count of the fresh fish ranged from  $0.35 \times 10^1$  cfu/g (Salmonella) to  $1.6918 \times 10^3$  cfu/g (Total Bacterial Count) and the smoked one ranged from  $0.5 \times 10^1$  cfu/g (Salmonella) to  $1.042 \times 10^3$  cfu/g (Total Bacterial Count). The fungal load of the fishes ranged between  $1.867 \times 10^2$  cfu/g for the smoked and  $4.860 \times 10^2$  cfu/g for the fresh ones. Statistically, there was no significant difference between the fresh and smoked fish with respect to microbial load at  $p < 0.05$ . The moisture content of the fresh fish was higher ( $74.48 \pm 4.52$ ) than that of the smoked fish ( $53.48 \pm 2.06$ ) just like the pH of the fresh ( $6.57 \pm 0.10$ ) and smoked fish ( $6.17 \pm 0.14$ ). The bacteria and fungi isolated from fish in the Creek road market ( $1550 \pm 265$  cfu/g,  $381.50 \pm 1.75$  cfu/g) were significantly higher/different from those of the mile 3 market ( $1262.50 \pm 310$  cfu/g,  $307.50 \pm 1.69$  cfu/g) and the mile 1 market ( $1287.75 \pm 6.15$  cfu/g,  $315 \pm 197$  cfu/g) at  $p < 0.05$ . *Bacillus subtilis*, *Krebsiella* spp, *Staphylococcus aureus* and *Streptococcus* spp were found only on smoked fish while *Acinetobacter* spp, *Corynebacterium* spp, *Flavobacterium* spp, *Enterobacter* spp and *Salmonella* species were found on fresh. Bacteria like *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Proteus* spp and *Serratia* spp were found on both fresh and smoked fish. Fungal species such as *Penicillium expansum*, *Aspergillus* spp, *Fusarium* spp, *Rhizopus stolonifer* and *Mucor piriformis* were found on both fresh and smoked fish. The microbial metrics or count showed that they all exceeded the permissible limits of the World Health Organization (WHO), Standard Organization of Nigeria (SON) and the National Environmental Standard Regulation Enforcement Agency (NESREA). The microbial count showed some level of contamination under the influence of pH and moisture. It is therefore crucial to implement strict hygiene and safety standards throughout the fish supply chain to mitigate these risks of consuming contaminated seafood.

**Key words:** Comparative Evaluation, Microbiological Quality, Public Health Risks, Fresh and Smoke Fish, Port Harcourt

## INTRODUCTION

Fish and fishery products are essential components of the human diet, providing a valuable source of high-quality protein, vitamins, minerals, and beneficial omega-3 fatty acids (FAO,2020). The consumption of fish and fish products has been associated with numerous health benefits, including reduced risk of cardiovascular diseases, improved cognitive function, and enhanced immune system (Domingo,2016, Sioen *et al.*,2007). Consequently, there has been a growing global demand for fish and fish-based products in recent years.

In Nigeria, the consumption of smoked fish is a widespread and popular practice, particularly in the southern region, including the city of Port Harcourt (Adeyemi and Osilalu,2019). Smoked fish is a preferred choice among consumers due to its distinct flavor, extended shelf-life, and perceived health benefits (Eyo,2001). However, the microbiological quality of smoked fish is a major concern, as the smoking process and subsequent handling and storage conditions can significantly impact the growth and proliferation of pathogenic microorganisms (Obemeta *et al.*,2011, Gram and Huss,1997).

Microbiological contamination of fish and fish products can pose significant public health risks to consumers, leading to the transmission of foodborne illnesses, such as salmonellosis, listeriosis, and staphylococcal food poisoning (Huss,1997,Omeiza *et al.*,2013). These pathogenic bacteria can be introduced during various stages of the production and distribution chain, including harvesting, processing, handling, and storage (ICMSF,1998). Moreover, the consumption of contaminated fish and fish products can result in severe gastrointestinal symptoms, hospitalization, and even fatalities in vulnerable populations, such as the elderly, young children, and immuno compromised individuals (Scallan *et al.*,2011,Nyarko *et al.*,2011).

While several studies have investigated the microbiological quality of smoked fish in different regions of Nigeria, there is limited research on the comparative assessment of the microbiological quality between smoked and fresh fish sold in the Port Harcourt metropolitan area (Adeyemi, & Osilalu, 2019,Odu, & Ukpoma,2013). This information is crucial for understanding the potential public health risks associated with the consumption of these fish products and developing targeted interventions to improve food safety.

Therefore, the present study aims to conduct a comparative assessment of the microbiological quality of smoked and fresh fish sold in selected markets in Port Harcourt City, Nigeria, and to evaluate the potential public health impact on consumers. The findings from this research will contribute to the existing knowledge base and provide valuable insights for policymakers, regulatory authorities, and the fish processing industry to enhance the microbial safety and quality of fish products in the study area.

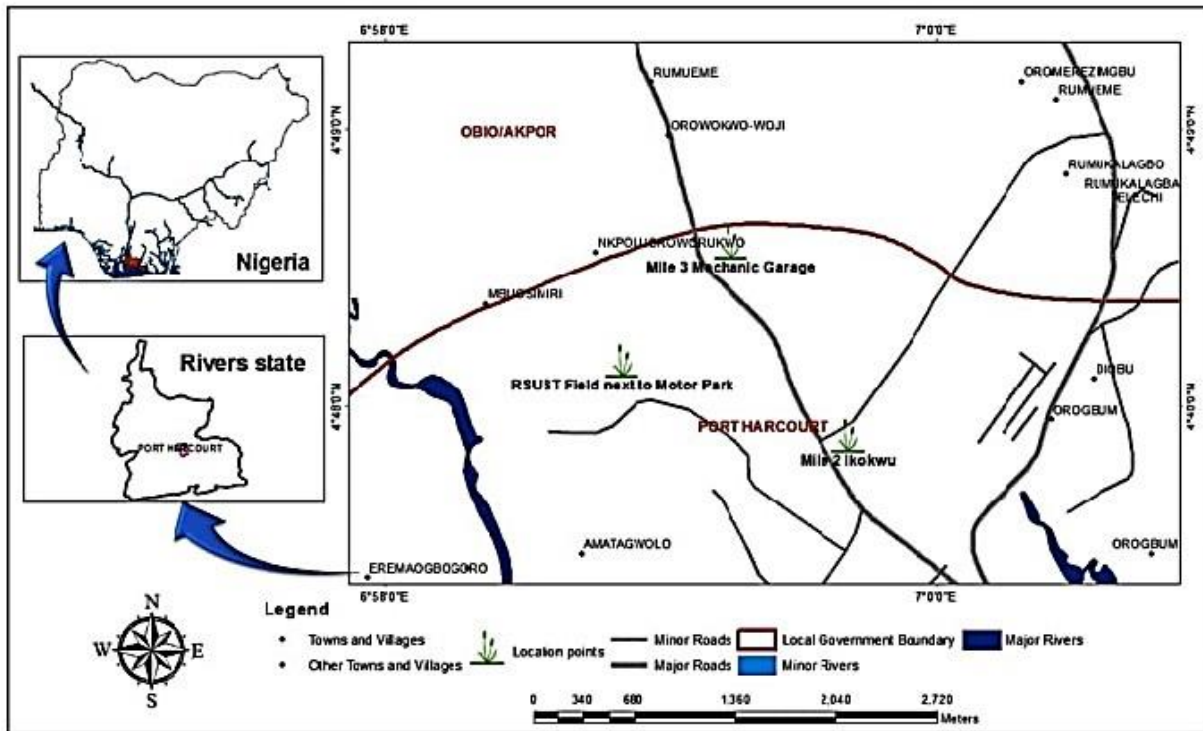
## Materials and Methods.

### Study Area and Sample Collection:

The study was conducted in Port Harcourt City, the capital of Rivers State, Nigeria. Port Harcourt is located in the southeastern region of Nigeria and serves as the capital of Rivers State. The city

is situated on the eastern bank of the Bonny River, approximately 40 kilometers inland from the Atlantic Ocean. Its geographic coordinates are approximately **4.8156° N latitude and 7.0495° E longitude (Figure 1)**. It is a major commercial and industrial hub in the southern region of the country, with a large population and thriving fish markets (Adeyemi and Osilalu,2019).Port Harcourt is surrounded by tropical rainforest vegetation, featuring a diverse range of plant species. The area is known for its dense foliage, including hardwood trees, shrubs, and various grasses. The rich biodiversity supports both terrestrial and aquatic ecosystems, particularly in the adjacent mangrove swamps.

A total of 120 fish samples of Silver catfish (*Chrysichthys nigrodigitatus*) were purchased from the three markets (Mile 3, Mile 1 and Creek Road) in Port Harcourt. These samples included 60 smoked and 60 fresh fish representing one of the most common fish type consumed in the study area (Eyo,2001). The fish samples were collected in sterile bags and transported to the laboratory for microbiological analysis within 2 hours of collection (Gram and Huss,1996).



**Figure 1: Map Showing the Sampling Locations in Port Harcourt**

### Microbiological Analysis:

Upon arrival at the laboratory, the fish samples were processed for microbiological examination. The skin, gills, and intestinal contents of the fish were aseptically homogenized, and serial dilutions were prepared using sterile saline solution (0.85% NaCl) (ICMSF,1998).

### **Enumeration of Total Viable Counts (TVC):**

To determine the total viable counts (TVC) of bacteria, aliquots of the appropriate dilutions were pour-plated onto Plate Count Agar (PCA) and incubated at 37°C for 24-48 hours. The number of colony-forming units (CFU) per gram of fish sample was then calculated (ICMSF,1998).

### **Isolation and Identification of Pathogenic Bacteria:**

For the isolation and identification of pathogenic bacteria, the fish samples were inoculated onto selective media, including *Salmonella-Shigella* (SS) Agar for the isolation of Salmonella and Shigella species (Obemeata *et al.*,2011), Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus species* (Huss,1997) and Eosin Methylene Blue (EMB) Agar for the isolation of *Escherichia coli* (Scallan *et al.*,2011).

The inoculated plates were incubated at 37°C for 24-48 hours. Presumptive colonies were further identified using standard biochemical and morphological tests, such as Gram staining, catalase, oxidase, and API identification systems (Omeize *et al.*,2011).

### **Fungal Enumeration**

Fungal counts were determined by inoculating 1 g of the homogenized sample onto Potato Dextrose Agar (PDA) plates, followed by incubation at 25°C for 5-7 days. The colonies were counted and expressed as CFU/g.

### **pH and Moisture Content Determination**

**pH Measurement:** The pH of each fish sample was measured using a calibrated pH meter. A 10 g sample was mixed with 90 mL of distilled water, stirred for 30 minutes, and the pH was recorded.

**Moisture Content:** Moisture content was determined by the oven-drying method. A 5 g sample was weighed and dried in an oven at 105°C until a constant weight was achieved. The moisture content was calculated as a percentage of the initial weight.

### **Statistical Analysis:**

The data collected from the microbiological analysis were subjected to appropriate statistical tests, including one-way analysis of variance (ANOVA) and post-hoc comparisons, to determine the significant differences in the microbiological quality between smoked and fresh fish samples. The level of statistical significance was set at  $p < 0.05$  (Nyarku *et al.*,2011).

### **Ethical Considerations:**

The study protocol was reviewed and approved by the Ethics Committee of the Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. Informed consent was obtained from the fish vendors before sample collection and the confidentiality of the data was maintained throughout the study (WMA,2013).

## RESULTS

The results of the study showed that the microbial count of the fresh fish was higher than that of the smoked fish with the fresh ranging from  $0.35 \times 10^3$  cfu/g (Salmonella) to  $1.6918 \times 10^3$  cfu/g (Total Bacterial Count) and the smoked one ranging from  $0.5 \times 10^1$  cfu/g (Salmonella) to  $1.0416 \times 10^3$  cfu/g (Total Bacterial Count) (Table1) respectively. The fresh fish also had higher fungal load ( $4.860 \times 10$ , cfu/g) than the smoked ones ( $1.8667 \times 10^2$  cfu/g) (Table1). Statistically, Table 2 showed that there was no significant difference between the fresh and smoked fish with respect to microbial load at  $p < 0.05$ .

The moisture content of the fresh fish was higher ( $74.48 \pm 4.52$ ) than that of the smoked fish ( $53.48 \pm 2.06$ ) just like the pH of the fresh ( $6.57 \pm 0.10$ ) and smoked fish ( $6.17 \pm 0.14$ ) Table 2)

**Table 1: Mean Value of Microbial Load of Fresh and Smoked Fish (*Chrysichthys nigrodigitatus*) in the Study Area**

Fish Type	Total Bacteria Count (CFU/g)	Coliform Count (CFU/g)	Salmonella (CFU/g)	Fungal Count (CFU/g)
Fresh	1691.83±191 ( $1.691 \times 10^3$ )	325.8±55 $3.258 \times 10^2$	1.63 $0.35 \times 10^1$	486.67±66.8 $4.86 \times 10^2$
Smoked	1041.6±276 ( $1.044 \times 10^3$ )	54.58±23 $5.458 \times 10^1$	0.50±0.55 $0.5 \times 10^1$	186.67±59.99 $1.867 \times 10^2$

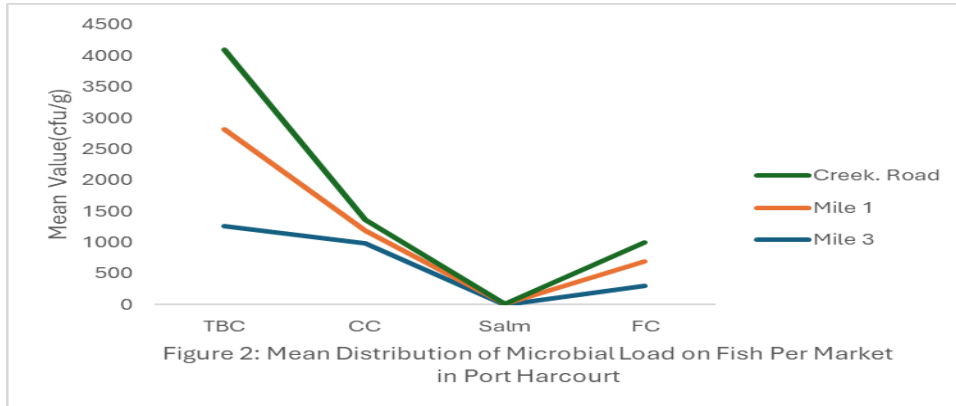
**Table 2: T-test for the Microbial Load of the Fresh and Smoked Fish in the Study Area**

Fish Type	Mean	SD	t	df	Sig(2-tailed)
Fresh	43.3417	646.80	1.634	5	0.163
Smoked	48.6350	70.51	1.690	5	0.152

**Table 3: pH Value and Moisture Content of Fresh and Smoked Fish in the Study Area**

Parameters	Fresh Fish	Smoked Fish
pH Value	<b>6.57±0.10</b>	<b>6.16±0.14</b>
Moisture Content	<b>74.48±4.52</b>	<b>53.48±2.66</b>

The bacteria and fungi isolated from fish in the Creek road market ( $1550 \pm 265$  cfu/g,  $381.50 \pm 1.75$  cfu/g) were significantly higher/different from those of the mile 3 market ( $1262.50 \pm 310$ , cfu/g,  $307.50 \pm 1.69$  cfu/g) and the mile 1 market ( $1287.75 \pm 6.15$  cfu/g,  $315 \pm 197$  cfu/g) at  $p < 0.05$  (Figure 2).



The result showed bacteria such as *Bacillus substilis*, *Krebsiella spp*, *Staphylococcus aureus* and *Streptococcus spp* were found only on smoked fish while *Acinetobacter spp*, *Corynebacterium spp*, *Flavobacterium spp*, *Enterobacter spp* and *Salmonella* were found on fresh fish (Table 4). Bacteria like *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Proteus spp* and *Serratia spp* were found on both fresh and smoked fish. Similarly, fungi such as *Penicillium expansum*, *Aspergillu spp*, *Fusarium spp*, *Rhizopus stolonifer* and *Mucor piriformis* were found on both fresh and smoked fish.

Table 5 showed the main values and the permissible limits of the national and international agencies of the microbial metrics. The mean values of the microbial metrics or count showed that they all exceeded the permissible limits of the World Health Organization (WHO), Standard Organization of Nigeria (SON) and the National Environmental Standard Regulation Enforcement Agency (NESREA). Total bacteria count in the fish was  $1366.75 \pm 408.293$  cfu/g against the permissible limit of  $<1000$  cfu/g for fresh and smoked fish while fungal count was  $336.67 \pm 167.947$  cfu/g against  $<100$  cfu/g.

**Table 5: Bacteria and Fungal Isolates on Fresh and Smoked Fish in the Study Area**

S/N	Bacteria Species	Fresh Fish	Smoked Fish	Fungal species	Fresh Fish	Smoked Fish
1	<i>Acinetobacta spp</i>	+	-	<i>Aspergillus expansium</i>	+	+
2	<i>Bacillus substilis</i>	-	+	<i>Fusarium spp</i>	+	+
3	<i>Corynebacterium spp</i>	+	-	<i>Mucor piriformis</i>	+	+
4	<i>Escherichia coli</i>	+	+	<i>Rhizopus stolonifera</i>	+	+
5	<i>Flavobacterium spp</i>	+	-	<i>Saccharomycus spp</i>	+	+
6	<i>Klebsiella spp</i>	-	+			
7	<i>Enterobacter spp</i>	+	-			
8	<i>Micrococcus luteus</i>	+	+			
9	<i>Pseudomonas aeruginosa</i>	+	+			
10	<i>Staphylococcus epidermidis</i>	+	-			
11	<i>Staphylococcus aureus</i>	-	+			
12	<i>Streptococcus spp</i>	-	+			
13	<i>Proteus spp</i>	+	+			

14	<i>Serratia spp</i>	+	+
15	<i>Salmonella spp</i>	+	-

**Key: Spp=species, +=present, - = Absent**

**Table 6: Overall Mean Value (SD), Minimum and Maximum Value and the Permissible Limits by Agencies**

Microbes	Mean	SD	Mini	Max	WHO	SON	NESREA
TBC	1366.75	408.293	750	2001	≤1000CFU/g	≤1000CFU/g	≤1000CFU/g
CC	190.17	147.295	17	410	Absent in 100g	Absent in 100g	Absent in 100g
Salm	2.00	1.954	0	6	Absent in 25g	Absent in 25g	Absent in 25g
Fungi	336.67	167.947	140	600	≤100cfu/g	≤100cfu/g	≤100cfu/g

**Key: TBC=Total Bacterial Count, CC=Coliform Count, Salm=Salmonella, WHO= World Health Organisation, SON=Standard Organisation of Nigeria, NESREA=National Environmental Standard Regulation Enforcement Agency**

## DISCUSSION

The observed difference microbial load in fresh than dried fish in the study could be attributed to the hygienic condition under which they were handled (Tiamigu *et al*; 2011). This is in line with the assertions by the researchers (Ieroi *et al*; 1998 Huss 1995, Mez-Guillen *et al*, 2002, Adebayo-Tayo *et al* 2009, FAO 2009, Sattar *et al* 2000) that maintaining high hygienic standard at every stage from harvesting to consumer handling is essential for controlling microbial load of both fresh and dried fish. The observed influence of moisture content and pit on the microbial load of the fresh and dried fish in their study is in agreement with the finding of Udochukwu *et al* (2016) in Benin city. Huss (1995) opined that high moisture content in fresh fish facilitate the growth of bacteria while Adebayo Tayo *et al* (2009) disclosed that low moisture content in fish reduce microbial loads in dried fish. According to Omojola (2005) moisture content and pH interact to influence the microbial ecology of fish.

The presence of the bacteria such as *Bacillus substilis*, *Klebsiella spp*, *Staphylococcus aureus* and *Streptococcus spp* on smoked fish in this study is in consonance with the observation of Udochukwu *et al* (2016) in Benin city. According to Adesiyun *et al* (2006) the presence of *Staphylococcus aureus* is of safety concern because they produce enterotoxins that lead to food poisoning and can be introduced into fish through contaminated hands, surfaces or processing equipment. Claucus and ward (1996) reported *Staphylococcus aureus* to occur naturally as microflora of fish and shellfish. Kosygin *et al* (1990) also reported *Bacillus substilis*, *Staphylococcus aureas* *Proteus mirabilis* *Klebsiella spp*, *Salmonella typical* and *Streptococcus spp* to be associated with smoked fish through human handlers, air and soil. Adebayo-Tayo *et al* (2009) opined that the presence of *Enterobacter spp* in the fish sample is a strong indication of faecal contamination which poses risks for food safety and public health. Efstathiou *et al*; (2011) also regarded *Enterobacter spp* as indicator organisms in seafood products. Ipki and offem (2008)

reported *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*, *Salmonella spp* from the gills, intestine and whole body of catfish *Clarias gariepinus* and seafood in Malaysia. The presence of *Aspergillus spp*, *Rhizopus sp*, *Fusarium spp* and *Penicillium spp* in smoked fish species could be attributed to reabsorption of moisture from the environment during storage which led to the growth of microorganism in addition to poor handling, processing and display (Christiana *et al*; 2010). The exceedance of bacterial and fungal counts in fish in this study could be primarily caused by poor hygiene, practices, inadequate processing, improper storage and consumer mishandling (Adebayo-Tayo *et al* 2009, Huss 1995, Efstathiou *et al*;2011). The health implication of consuming contaminated fish ranges from acute food borne illness to long-term health risks.

### CONCLUSION AND RECOMMENDATION

The results of this study with the observed presence of pathogenic organisms and the exceedance of the microbial load above the permissible limits of the standard agencies such as WHO, SON and NESREA in the fresh and smoked seafood (fishes) indicates clear sign of contamination from several sources. It is therefore crucial to implement strict hygiene and safety standards throughout the fish supply chain to mitigate these risks of consuming contaminated seafood.

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