

Mycoflora and Public Health Risks of Smoked Fish Sold in Port Harcourt Markets, Nigeria

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Abstract Fish is a preferred source of protein globally, especially in developing countries like Nigeria. It is a savoured protein source in the Niger Delta, including Port Harcourt. Smoking is used to preserve fish by reducing its moisture content with a view to improved shelf life. This study aimed at determining the Mycoflora and the Public Health risks of smoked fish sold in Port Harcourt Markets. A total of 54 fish samples were collected from three strategic markets; Mile one, Oil Mill and Creek Road markets. Fish collected consists of 6 different species; *Gadus morhua*, *Pseudotolithus typhus*, *Lutjanus goreensis*, *Ethalmosa fimbriata*, *Pseudotolithus senegalensis* and *Dasyatis pastinaca*. All samples were grouped accordingly. Mycological study of fish samples was done using standard methods on Sabouraud Dextrose Agar. There was a significant difference in the mycoflora counts of smoked fish from different markets ($p < 0.05$). Fungal load ranged from $1.23 \pm 0.08 \times 10^3$ sfu/g in *Lutjanus goreensis* to $8.89 \pm 0.10 \times 10^3$ sfu/g in *Gadus morhua*, at Creek road market. From Mile 1 market, *Lutjanus goreensis* still hosted the highest population of $13.25 \pm 0.7 \times 10^3$ sfu/g and *Dasyatis pastinaca* had the least; $0.66 \pm 0.01 \times 10^3$ sfu/g. At Oil mill market, *Ethalmosa fimbriata* hosted $13.23 \pm 0.47 \times 10^3$ sfu/g while *Gadus morhua* had $0.77 \pm 0.02 \times 10^3$ sfu/g. The fungal load in all fish from all three markets were significantly high for food and calls for attention. Nine fungal genera; *Saccharomyces* spp, *Rhizopus* spp, *Penicillium* spp, *Mucor* spp, *Fusarium* spp, *Cladosporium* spp, *Candida* spp, *Absidia* spp and *Aspergillus* spp, were isolated. All six fish species studied recorded more than 50 % occurrence of fungal species in all the markets. The mycoflora of smoked fish sold in Port Harcourt markets suggest significant public health risks. The need for improved storage and handling of this important protein source is high towards reduced public health risk. Proper preparation method, such as boiling, is strongly advocated.

Keywords: Mycoflora, public health, risks, smoked fish, port harcourt, markets

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1. Introduction

The Niger Delta Nigeria is home of majority of Nigeria's water bodies [1]. As expected, sea food forms a major part of the diet in this region. Port Harcourt is the current capital of Rivers State, a prominent state in the Delta. A travel through the markets in this oil-rich town, reveals a variety of sea foods in the markets. Prominent among these sea foods is fish of various sizes, species and display.

Fish is an important sea food of choice globally [2,3,4,5]. Presently, fish is quoted to constitute 60% total protein in humans [5]. It has been a preferred protein source over the years. The choice of fish as a protein source is owed to certain desirable features [2,4]. Fish is easy to digest, cheap, rich in protein, vitamins and minerals [5,6,7]. Further, the presence of constituents, like omega 3 fatty acid, make fish a preferred protein source [5]. The presence of only 10% cholesterol in fish has also made it a desired flesh protein [7]. Fish consumption has

increased because it defiles health, cultural as well as socio-economic barriers [2,6].

Fish is highly perishable [5], in fact the most perishable of muscle foods [2]. Thus, the need for proper fish preservation methods [2,5]. Smoking is a popular preservation method [8]. Smoked fish constitutes up to 45% of all fish consumed in Nigeria [7]. Preferred for convenience and the resulting aroma it leaves on the fish, smoking has been used widely in fish preservation. Primarily, smoking reduces the moisture content of food and thus limiting the activities of spoilage microorganisms [5]. Though very advantageous, smoking has some side effects such as inclusion of Polycyclic Aromatic Hydrocarbons (PAHs) in the preserved food [9].

Numerous studies have questioned the public health safety of smoked fish [2,5,7]. Fish at the point of harvesting possess a notable microbial load in various body parts [3,5]. These studies have isolated microorganisms posing health hazards from smoked fish. The microbial population that contaminates these fish depend on numerous factors such as season [5,7]. The presence of

these microorganisms could pose numerous public health risks. Further, smoking as a method of fish preservation could introduce polycyclic aromatic hydrocarbons [9] and yet fail to prevent microbial proliferation [5]. These PAHs have been implicated for various diseases of humans that could lead to mortality.

Food spoilage was not ascribed to fungi previously but fungal species have been shown responsible for spoilage recently [7]. The Mycoflora sums up the total fungal population in a sample. The Mycoflora of smoked fish could pose public health risk(s), especially considering fish consumption method and preparation. Studies have isolated numerous fungi from smoked fish [5,6,7,8]. Most of these fungi are known to have public health risks based on certain pathogenic attributes. Some fungi produce aflatoxins known to pose severe consequences including being carcinogens and damage organ [7]. Aflatoxins are teratogenic, nephrotoxic, immunotoxic and mutagenic [10]. They have been linked to cases of hepatitis especially in developing countries like Nigeria [5,7]. Nigeria has been described as endemic for hepatitis virus with up to 13% having Hepatitis B and 8 % having Hepatitis C [11]. Several episodes of epidemic and high mortality have been linked to fungal toxins [10]. Further, the immunosuppressing effects of aflatoxins could expose humans to infections such as Human Immunodeficiency Virus (HIV) and tuberculosis [12].

Smoked fish is considered safely preserved and consumed variously. In Nigerian cities, including Port Harcourt, smoked fish is integral to most of delicious dishes. It has been consumed variously; cooked and uncooked. The method of consumption could further bring the health risks to fore, especially when consumed raw. Although preserved and apparently safe from spoilage, smoked fish sometimes appear damp and with moulds. The presence of moulds on smoked fish raises some questions as to the mycological status and consequent public health risk(s) of smoked fish. Numerous studies have successfully established public health risks associated with smoked fish in Nigerian markets [2,5,7,9]. The present study therefore, was aimed at studying the Mycoflora of smoked fish in Port Harcourt markets as a way of establishing the public health risk(s) associated with smoked fish consumption.

2. Materials and Methods

2.1. Study Area

The study area was Port Harcourt, Rivers State. Three (3) popular markets were chosen for the present study. Fish samples were obtained from Mile One, Oil Mill and Creek Road Markets, on coordinates 4.7918°N, 6.9986°E; 4.5121°N, 7.352°E and 4.7582869°N, 7.0208996°E respectively. Markets were considered representative of the entire metropolis and usually the major source of smoked fish in Port Harcourt.

2.2. Sample Collection

A total of 54 smoked fish samples consisting six different species: *Gadus morhua* (stock fish), *Pseudotolithus typhus* (Croaker), *Lutjanus goreensis* (Red snapper), *Ethalmosa*

fimbriata (Bonga fish), *Pseudotolithus senegalensis* (Broke marriage) and *Dasyatis pastinaca* (Sting ray) were purchased from the various markets and transported in clean polythene bags to the laboratory for analysis. Each group (market) consisted of eighteen (18) samples used in the study.

2.3. Mycological Studies

Study of the Mycoflora of smoked fish was performed aseptically. Commercially available Sabouraud Dextrose Agar (SDA) (Biolab Laboratories, Hungary) was used. All dilutions were done in sterile normal saline. Standard plate count as in Cheesbrough (2006) [13] was used for isolation and enumeration of fungi.

For each set of 18 market samples, 10 g of smoked fish was weighed onto clean aluminium foil. Then the samples were macerated in 90 ml sterile normal saline using warring blender to give 10^{-1} dilution as in previous studies [7]. The mixture was diluted serially up to 10^{-4} and 0.1 ml of respective solutions pipetted into sterile petri dishes. Then molten SDA, containing penicillin and streptomycin, was poured and allowed to solidify. All set up, in triplicates, were incubated at 28 °C for 5 days. Colonies were then subcultured to obtain pure colonies used for identification. Identification was done using morphological and microscopic features (wet mount) with reference to Practical Mycology Manual for Fungi Identification [7].

2.4. Data Analysis

Analysis of Variance (ANOVA) was used to test all data for significance ($p < 0.05$) using SPSS version 23. Where differences occurred, Duncan's multiple range test (DMRT) was used to separate the means.

3. Results

A study of the Mycoflora of smoked fish showed numerous fungal genera in all three markets. All six fish species studied, presented with significant fungal load. In creek road market, the highest fungal load ($8.89 \pm 0.10 \times 10^3$ sfu/g) was recorded in *Lutjanus goreensis* while *Gadus morhua* had the least load ($1.23 \pm 0.08 \times 10^3$ sfu/g) (Table 1). In Mile 1 market, *Lutjanus goreensis* still recorded the highest fungal load ($13.25 \pm 0.70 \times 10^3$ sfu/g) while *Pseudotolithus typhus* had the least ($0.59 \pm 0.06 \times 10^3$ sfu/g). However, a look at results from Oil mill market showed that *Ethalmosa fimbriata* had the highest load ($13.23 \pm 0.47 \times 10^3$ sfu/g) with the *Gadus morhua* presenting the least load ($0.77 \pm 0.02 \times 10^3$ sfu/g) as in Creek road market.

Marked difference in the Mycoflora of the fish in various markets was recorded. Obviously, there was a significant difference ($p < 0.05$) in the Mycoflora of all fish studied across the markets apart from *Gadus morhua*, where difference was noted only between Creek road and other markets (Table 1).

Further, the percentage occurrence of fungal isolates was studied. Again, an interesting pattern in the occurrence of fungal species was recorded. Nine fungal

genera, including *Saccharomyces*, *Rhizopus*, *Penicillium*, *Mucor*, *Fusarium*, *Cladosporium*, *Candida*, *Absidia* and *Aspergillus* species were consistently present in all three market samples. Of the nine genera isolated, *Aspergillus* spp. (38.33%) closely followed by *Penicillium* spp. (26.67%) was most abundant in all markets (Figure 1). The least abundant species isolated across the markets however varied. They were *Cladosporium* spp. (1.67%) in Oil mill, *Mucor* (2.82%) in Creek road and *Rhizopus*,

Mucor and *Cladosporium* (1.89% each) in Mile one market.

The distribution of Mycoflora on the fish species was also studied and recorded. Interestingly, none of the fish species hosted all nine fungal species. The highest fungal presence was noted in *Ethalmosa fimbriata* (Bonga fish), with all species apart from *Candida tropicalis* present (Table 2). Otherwise, all other fish species studied presented with at least five of all nine fungal species.

Table 1. Fungal Populations ($\times 10^3$ sfu/g) of smoked fishes in the various markets in Port Harcourt

Markets	<i>Gadus morhua</i>	<i>Pseudotolithus typhus</i>	<i>Lutjanus goreensis</i>	<i>Ethalmosa fimbriata</i>	<i>Pseudotolithus senegalensis</i>	<i>Dasyatis pastinaca</i>
Creek road	1.23 \pm 0.08 ^b	1.51 \pm 0.09 ^b	8.89 \pm 0.10 ^a	1.72 \pm 0.07 ^b	1.57 \pm 0.07 ^a	1.72 \pm 0.06 ^b
Mile 1	0.87 \pm 0.21 ^a	0.59 \pm 0.06 ^a	13.25 \pm 0.70 ^c	0.73 \pm 0.06 ^a	2.63 \pm 0.15 ^b	0.66 \pm 0.01 ^a
Oil mill	0.77 \pm 0.02 ^a	7.53 \pm 0.25 ^c	11.34 \pm 0.65 ^b	13.23 \pm 0.47 ^c	3.59 \pm 0.15 ^c	3.58 \pm 0.04 ^c

*means with the same superscript along the columns are not significantly different ($p < 0.05$).

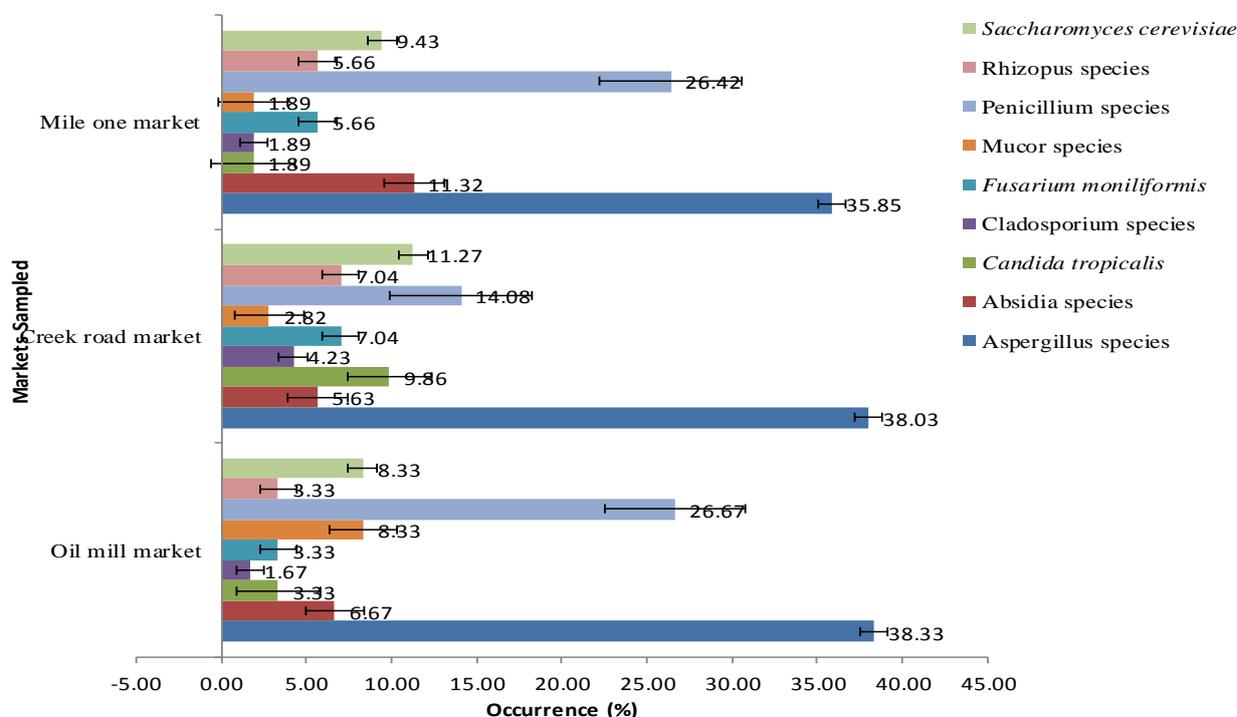


Figure 1. Percentage occurrence of Fungal isolates in the different markets sampled

Table 2. Distribution of fungal isolates in the different fishes sampled

Fungal Isolates	<i>Gadus morhua</i>	<i>Pseudotolithus typhus</i>	<i>Lutjanus goreensis</i>	<i>Ethalmosa fimbriata</i>	<i>Pseudotolithus senegalensis</i>	<i>Dasyatis pastinaca</i>
<i>Aspergillus species</i>	+	+	+	+	+	+
<i>Absidia species</i>	-	-	-	+	-	+
<i>Candida tropicalis</i>	+	-	+	-	+	-
<i>Cladosporium species</i>	+	+	-	+	+	-
<i>Fusarium moniliformis</i>	+	+	+	+	-	+
<i>Mucor species</i>	-	-	+	+	+	+
<i>Penicillium species</i>	+	+	+	+	+	+
<i>Rhizopus species</i>	+	+	-	+	-	-
<i>Saccharomyces cerevisiae</i>	+	-	+	+	-	-

Legend: + = present; - = absent.

4. Discussion

The fungal population in the smoked fish species in this study varied according to the location of the market and were significantly different ($p < 0.05$). The highest fungal load was recorded in *Ethalmosa fimbrata* ($13.23 \pm 0.47 \times 10^3$ sfu/g) while the least was recorded in *Dasyatis pastinaca* ($0.66 \pm 0.01 \times 10^3$ sfu/g) and *Gadus morhua* ($0.77 \pm 0.02 \times 10^3$ sfu/g). These results are however comparable to that of previous studies conducted in other parts of Nigeria [5,7,14]. Although the safe fungal load for food is at $< 10^6$ cfu/g [22], the health risk exposed by the present study is enormous. This is based on aflatoxin production and tolerable limit in humans ranging from less than 1 ng/kg body weight in developed countries to about 100 ng/kg body weight in Africa [12]. Several factors have been highlighted to support fungal infestation of fish. Some of the factors include temperature, humidity and handling [5]. Similarly, these factors also enhance aflatoxin production [10]. More worrisome, is the presence of these factors in the study area. Proper insight into the preservation processes leaves a lot to be desired. Harvested fish are preserved in many unhygienic ways due to its high perishability. Efforts are made to keep the temperature at safe levels. This has been achieved by using damp sack to cover as well as mixing with wet weed or green grass [15]. Although for good reasons, these handling practices could increase the Mycoflora of fish. The high Mycoflora in all markets could be blamed on hygiene conditions of all the markets.

Further, smoking is a method of fish drying that applies between 20°C and 30°C [16,17]. This temperature will not deter but may even encourage fungal proliferation as well as toxin production. Fungal growth temperature in the present study was 28°C. This is within the range of temperature obtainable in the traditional drying method. Obviously, this could explain the high fungal load of the fish studied. Also, aflatoxin production is favoured at temperatures between 24°C to 28°C [10]. Previous studies have reported that although smoking is seen as a good preservation method, the resulting fish do not have a good shelf life [7]. Shelf life prolongation is a key consideration in choosing a preservation method. More improved drying methods have been reported with better results and improved shelf life [16,17]. Such methods require more sophisticated procedure in Kilns, using higher temperatures and thus, better results. Fungi are spore-forming and these spores are resistant to heat [5]. These spores are implicated for colonisation and spoilage of dry materials such as smoked fish. Spore propagation could be fostered the more by the poor hygiene practices obtainable in the markets studied. The present study as well as previous studies has noted the display of fish close to dirty drainage gutters in markets leading to contamination of smoked fish [2,5,7,15].

Apart from drying procedure, handling has also been blamed for smoked fish contamination [2,3,5,7,18]. Specifically, the method of displaying these fish may not be proper. Display of fish for sale is mostly done in trays with no allowance for proper aeration. This could increase humidity and thus fungal proliferation as well as aflatoxin production. The study by Adebayo-Tayo *et al.* [7], also

highlighted the crowding of fish on trays for fungal infestation. Further, Port Harcourt is a humid city [19]. Naturally, this could have availed the needed humidity for fungal growth.

The lowest fungal population recorded in *Gadus morhua* (the normal stock fish) may be due to the method of drying in more regulated Kilns before importation. Apparently, the stock fish has lower proliferation requirements for fungal colonisation. This would explain the significant reduction in fungal load. Conversely, other smoked fish samples are dried in the local Kilns. These drying apparatus may not be able to achieve the temperatures of the more sophisticated Kilns. Thus, fungal load is higher in these locally preserved fish samples.

The Isolation of nine (9) fungal species (including, *Saccharomyces*, *Rhizopus*, *Penicillium*, *Mucor*, *Fusarium*, *Cladosporium*, *Candida*, *Absidia* and *Aspergillus*) in this study calls for concern as to the efficacy of the usual local drying methods and the mycological safety of smoked fish. Although fungal species occurred in varying frequencies, *Aspergillus* spp. (38.33%) closely followed by *Penicillium* spp. (26.67%) was most abundant in all markets. This finding is similar to those of other researchers [5,7,14], although they failed to isolate *Candida tropicalis* and *Absidia* spp. Fungal growth is supported by adequate moisture beyond 16 % [5,7,20]. Fish preservation by drying aims to remove up to 90 % moisture from the food and mould proliferation is hampered at 10 % moisture [5]. Total Mycoflora load in all fish samples in all markets were high and a source of concern considering their toxin production abilities [12].

The Mycoflora of smoked fish sold in Port Harcourt Markets suggest public health risks. Certain fungal species such as *Aspergillus* spp. and *Penicillium* spp have been implicated for public health risks [7]. *Aspergillus* and *Penicillium* species have been associated with the production of toxins including aflatoxins and ochratoxins leading to mortality [5,7,20]. With the level of fungal contamination in smoked fish, consumers may also be ingesting these fungal species as well as toxins and metabolites [5,7]. Most likely, the ingestion of these toxins will be above the tolerable dose per body weight per day [12].

5. Conclusion

Smoked fish sold in Port Harcourt presents with high fungal load and could pose significant public health risks. The use of more regulated, sophisticated Kilns would improve the quality of the resulting smoked fish. Further, it is important that fish handlers are educated to imbibe a quality assurance process in the model of the Hazard Analysis Critical Control Points (HACCP). Smoked fish should be adequately cooked prior to consumption to reduce public health risks.

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