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Determination of fungal contamination of retailed smoked-fish sold within Owerri metropolis, Imo State, Nigeria

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ABSTRACT

The consumption of smoked-fish usually from the open shelf in most communities of the developing countries has raised some health related concerns. As one of the common sources of protein available to man, fish is highly consumed due to its lower cholesterol content and price. So it forms a rich protein source for both poor and rich. This research enumerated the fungal load of retailed smoked-fish sold within Owerri metropolis, Imo State, Nigeria. A total of thirty six fish samples which comprised nine fish species such as *Ethmalosa fimbrata* (Bonga fish), *Clarias garepinus* (Mud cat fish), *Ilisha Africana* (West African shad) *Alestes nurse* (Silverside fish), *Chrysichthys nigrodigitatus* (Silver catfish), *Gymnallabes typus* (Catfish), *Calamoichthys calabaricus* (Rope fish), *Schilbe uranoscopus* (Butter fish) and *Cynoglossus browni* (Sole) were randomly purchased from markets in Owerri. The fish samples were inoculated onto Sabouraud dextrose agar plates incorporated with 0.5mg/ml of chloramphenicol. The fungal isolates were identified using standard methods Thirty four fungal organisms made up of four genera: *Aspergillus* species, *Penicillium* species, *Fusarium* species and *Rhizopus* species were isolated from the fish samples. *Aspergillus flavus* 8 (23.53%), *Aspergillus fumigatus* 7 (20.58%) and *Rhizopus* species 7 (20.58%) were the dominant mycofloral in decreasing sequential order. *Aspergillus niger*, *Fusarium* species and *Penicillium* species occurred in less frequency 4 (11.77%). *Aspergillus flavus* was the most dominant mycofloral. *Aspergillus* species are known to produce aflatoxins which are carcinogenic (causing hepatoma-cancer of the liver), acute hepatitis, reduced red blood cell and decreased immune system in man. *Fusarium* species was reported to produce fumonisin toxin and *Penicillium* produces penicillic acid. Prolonged intake of smoked-fish with these metabolites may constitute potential public health hazard. It is recommended that smoked-fishes be processed, stored and distributed under safe hygienic condition and good sanitary practices in order to prevent fungal contamination.

Keywords: smoked-fish, aflatoxins, carcinogenic, fumonisin toxin, penicillic acid, public health

1. INTRODUCTION

Fish is an important source of dietary protein, vitamins and minerals and from the economic stand-point; it is a source of income to many people in developing countries. In African, some 5 percent of the population about 35 million people depend wholly or partly on the fisheries sectors, mostly artisanal fisheries, for their livelihood. Fish is highly perishable due to its high moisture and fat content; it has relatively 10% calories content hence its role in nutrition is recognized [1]. Fish and fish products constitute more than 60% of the total protein intake in adults especially in rural areas [2]. They are widely accepted on the menu card and from a much-cherished delicacy that cuts across socio-economic, age, religious, and educational barriers [2]. Fish flesh is one of the best sources of protein. Its flesh is tender due to bundles of muscles fibers, which are held together by fibrous materials when heated. It contains most of the important amino acids, particularly, lysine, methionine and tryptophan that are lacking in plant proteins. Apart from its food value, fish has been reported to possess medicinal values, such as, in the amelioration of asthma, arthritis, coronary heart diseases, goiter and cancer. It is better digested than beef or other types of protein [3]. In Nigeria, fish is eaten fresh, preserved or processed.

Fish is an extremely perishable food commodity as it is highly susceptible to autolysis (the destruction of a cell through the action of its own enzymes), rancidity (which occurs especially in oily fish such as hering, mackerel and sardines as a result of oxidation of fat which creates unpleasant odors and flavors), hydrolysis and microbial spoilage [4] therefore, requiring prompt processing and preservation to avoid spoilage. Various traditional methods are employed in preservation and processing of fish for consumption and storage. These include smoking, drying, freezing, salting, frying and fermentation. In Nigeria, smoking is the most widely practiced method, they are highly desirable because of it enhanced flavor and texture in fish in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations. Practically all species of fish available in the country can be smoked and it has been estimated that 70-80 percent of the domestic marine and fresh water catch is consumed in smoked form. Smoking demonstrates a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content [5]. Smoking-drying method used in Nigeria requires low capital, investment and it is conducted in fisherman camps and fish processing centers in traditional smoking kilns of clay, cement blocks, drums or iron sheets. Smoked-fishes can be kept for 2-4 weeks market stalls with poor storage facilities.

Several fungal organisms associated with the contamination of dried smoke fishes, such as *Aspergillus*, *Penicillum*, *Zygomycetes* and yeasts are known to be opportunistic pathogens [6] causing varying degrees of health problems to both animals and man. Swaminathan and Sparling [7] reported that numerous pathogenic agents isolated from different types of fish were toxigenic, and their toxins retained in fish flesh even after salting and storage periods which causes serious systemic dysfunctions and public health hazards [8]. Consumption of smoke-dried fish without further cooking is common in Nigeria. Corroborating the report of [7, 9] pointed out that most mycotoxins are stable compounds that are not destroyed during food

processing or home cooking, and several cases of human gastroenteritis, severe diarrhoea and food poisoning outbreaks have been recorded after smoked fish consumption [8]. Despite the numerous reports on the fungal contaminants of smoke-dried fish in Nigeria and its public health implications, documented reports and studies on the mycology of dried smoke fish in Owerri, Imo State are scarce. It is therefore important to determine the fungal contamination of dried smoked fish as it is of public health importance.

2. MATERIALS AND METHODS

Sample Collection

Thirty six samples of dried smoked-fish for this study were randomly purchased from fish retailers at Eke-onunwa market in Owerri, Imo State. The species of the smoked-fish samples are *Ethmalosa fimbriata* (Bonga fish), *Clarias garepinus* (Mud cat fish), *Ilisha Africana* (West African shad), *Alestes nurse* (Silverside fish), *Chrysichthys nigrodigitatus* (Silver catfish), *Gymnallabes typus* (Catfish), *Calamoichthys calabaricus* (Rope fish), *Schilbe uranoscopus* (Butter fish) and *Cynoglossus browni* (Sole). The samples were subsequently packed in sterile polyethylene bags, labeled accordingly and transported to the department of microbiology laboratory, Imo State University for microbiological analysis.

Preparation and Inoculation of samples

The fish samples were blended and kept in airtight sterile containers. Five-fold serial dilutions were prepared to 10^5 with one gram (1g) of each of the sample in 9ml sterile water [10]. One milliliter of each dilution was dispensed in sterile Petri dishes and molten Sabouraud dextrose agar incorporated with 0.5mg/ml of chloramphenicol were added to the Petri dishes. The plates were allowed to solidify and were incubated at 25°C for 3 days. The colonies observed were subcultured on SDA without antibiotics to obtain pure culture of each isolate and to enhance proper identification.

Identification of fungal isolates

The fungal isolates on SDA were identified based on macroscopic and microscopic morphological features and biochemical tests using standard methods [10-12]. The growth rate, color, texture, colonial morphology and diffusible pigments of the fungal isolates were examined macroscopically. The morphology of the spores and hyphae were examined microscopically. A drop of lactophenol cotton blue stain was placed on a clean grease-free slide. A small fragment of woolly or powdery colony was picked using a sterile needle and teased in the stain until a homogenous blue mixture of stain and culture was obtained. A clean cover slip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation was examined using X10 and X40 objectives of the microscope respectively. The appearance of macroconidia, microconidia and hyphae were observed.

3. RESULTS

This study was aimed at determination of fungal contamination of retailed smoked-fish sold within Owerri metropolis. Table 1 shows the cultural characteristics of fungal isolates.

A variety of fungal species were isolated from the fish samples sold within the study area. They include: *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus* species, *Aspergillus niger*, *Fusarium* species and *Penicillium* species.

Table 1. Cultural characteristics of fungi isolated from fish samples sold within Owerri metropolis

Fungal isolates	Cultural characteristics
<i>Aspergillus flavus</i>	Yellow-green, powdery and pale, yellow on reverse
<i>Aspergillus fumigatus</i>	Blue- green, powdery and pale yellow on reverse
<i>Rhizopus</i> species	White-milk colonies which later turns black
<i>Aspergillus niger</i>	White- black reverse turning pale yellow
<i>Fusarium</i> species	Growing wooly-cottonly lemon and yellow
<i>Penicillium</i> species	Largely fluffy white colonies almost covering the whole surface

The distribution of fungi isolated from fish samples sold within Owerri metropolis is shown in Table 2. *Ethmalosa fimbriata* (Bonga fish) recorded the highest frequency of isolation and percentage of fungi [5 (14.72%)], followed by *Clarias garepinus* (Mud cat fish), *Alestes nurse* (Silverside fish), *Chrysichthys nigrodigitatus* (Silver catfish), *Gymnallabes typus* (Catfish) and *Cynoglossus browni* (Sole) which recorded [4 (11.77%)] each. *Ilisha Africana* (West African shad), *Calamoichthys calabaricus* (Rope fish) and *Schilbe uranoscopus* (Butter fish) recorded the least frequency of isolation and percentage of fungi [3 (8.81%)].

Table 2. Distribution of fungi isolated from fish samples sold within Owerri metropolis

Samples	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Fusarium</i> species	<i>Rhizopus</i> species	<i>Penicillium</i> species	Total (%)
<i>Ethmalosa fimbriata</i>	+	+	+	-	+	+	5 (14.72)
<i>Clarias garepinus</i>	+	-	+	+	+	-	4 (11.77)
<i>Ilisha africana</i>	+	+	-	+	-	-	3 (8.81)

<i>Alestes nurse</i>	+	+	+	-	+	-	4 (11.77)
<i>Chrysichthys nigrodigitatus</i>	+	+	-	+	-	+	4 (11.77)
<i>Gymnallabes typus</i>	-	+	+	-	+	+	4 (11.77)
<i>Calamoichthys calabaricus</i>	+	+	-	-	+	-	3 (8.81)
<i>Schilbe uranoscopus</i>	+	+	-	-	+	-	3 (8.81)
<i>Cynoglossus browni</i>	+	-	-	+	+	+	4 (11.77)
Total	8 (23.53)	7 (20.58)	4 (11.77)	4 (11.77)	7 (20.58)	4 (11.77)	34 (100)

Key: + = Presence of fungi isolate - = Absence of fungi isolate

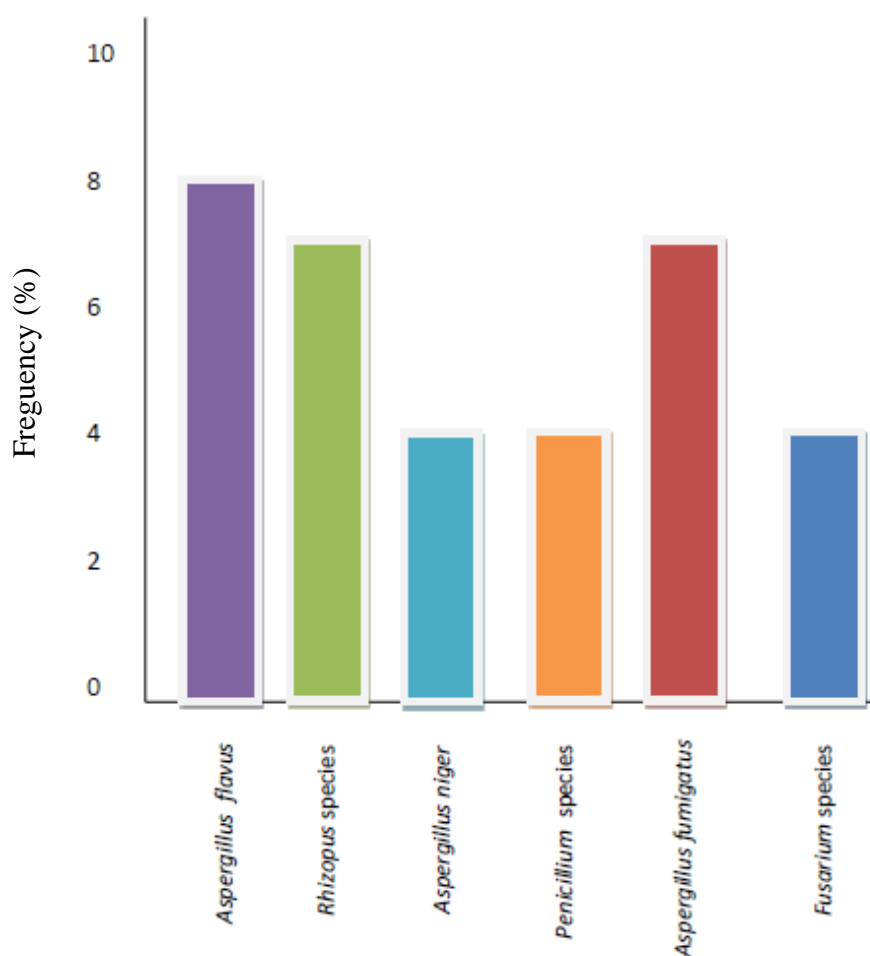


Fig. 1. The frequency of occurrence of fungi associated with retailed smoked-fish samples sold within Owerri metropolis, Nigeria

The bar chart (Fig. 1) shows the frequency of occurrence of fungi contamination of retailed smoked-fish sold within Owerri metropolis. A total of 34 fungal organisms were isolated from the 9 fish species examined. *Aspergillus flavus* 8 (23.53%), *Aspergillus fumigatus* 7 (20.58%) and *Rhizopus* species 7 (20.58%) were the dominant mycofloral in decreasing sequential order. *Aspergillus niger*, *Fusarium* species and *Penicillium* species occurred in less frequency 4 (11.77%).

4. DISCUSSION

The results of this study showed fungal contamination of dried smoked fishes. Similar findings by [8, 13-17] reported in similar mycological studies on smoke-dried fish sold in markets in different parts of Nigeria concur to the findings of this study. The fungal contamination associated with smoked fishes could be from the environment in which the fishes are harvested and not specific to a particular species [18]. In this study, the samples which were smoked on charcoals or wood barbecue were either displayed on dirty trays, open containers or untidy tables in the markets for sales. Osibona *et al.* [17] observed in their study that storage containers play a vital role in the preservation and shelf-life of smoked dried fish, and recommended the use of airtight storage containers for smoked fish. According to [19], processed fish are easily contaminated with microorganisms if post-processing handling is not properly done under hygienic conditions. Also, the quality of smoked fishes is dependent on several factors including, the preparation of the raw materials, the nature of wood and type of the smoking procedures employed [20]. During the smoke-drying period, [21] and [1] cited by [8] pointed out that smoking kilns used in artisanal fishery and the overloading of the fish in the trays leads to improper processing which in turn encourages fungal contamination. Thus the growth of fungi in smoke-dried fish can be affected by the storage method employed. From the results of this study, *Aspergillus flavus* was the most dominant mycofloral. According to [22], *Aspergillus flavus* and *Aspergillus fumigatus* are probably the most notorious of the common isolate because of their high potentials in producing *aflatoxins*, which destroyed the liver and kidney in man resulting to death and also causes acute hepatitis (*Aflatoxicosis*). The presence of *Aspergillus flavus* and *Aspergillus fumigatus* in the studied smoked fish samples is of great health concern because of their mycotoxigenic potential. The occurrence of *Aspergillus*, *Rhizopus*, *Penicillium* and *Fusarium* species could be due to the fact that during storage, the fish products reabsorbed moisture from the environment which then supports the growth of micro-organisms in addition to contamination during processing, handling and displayed on the market stalls. Many fungal organisms associated with the contamination of smoke-dried fish sold in markets in Nigeria have been reported in different studies [8, 13-16, 23-33]. These are serious safety concerns related to the consumption of raw fish and shellfish because of the presence of biological (bacteria, virus and parasites) and chemicals (biotoxins) hazards. These hazards are present in fish and shellfish pre-harvest and are therefore, difficult to control.

5. CONCLUSION AND RECOMMENDATIONS

This study had shown that smoked-fishes sold in markets in Owerri metropolis, Imo State are contaminated with spoilage fungi. The hazards related to contamination, recontamination

or survival of biological hazards during processing of smoked-fishes could be controlled by applying good manufacturing and hygienic practices.

Based on these findings, it is recommended for the use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture. Fish retailers should showcase the commodity in closed transparent containers instead of displayed it in open trays to minimize contamination. Consumers bargaining for the commodity should avoid making direct contact with the commodity using bare hands to curtail cross contamination. People should properly cook their fish before eating even when it is smoked to avert food poisoning. Appropriate authorities; such as National Agency for Food and Drugs Administration and Control (NAFDAC) should enforce proper environmental condition of our food handlers as it concerns the smoking factories and ensure constant power supply in order encourage effective preservation of fish prior to smoking. Also, the hygienic conditions of the markets where these fishes are sold and the hawkers that carry the fishes from one place to another must be ascertained before authorizing them to handle public foods.

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