



## Occurrence of Aflatoxigenic Fungi in Smoke-dried Fish Sold in Jos Metropolis

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors MOJ and SEA designed the study, performed the statistical analysis, wrote the protocol and authors HSD and MOJ wrote the first draft of the manuscript and managed literature searches. All the authors managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

This research was conducted to determine the occurrence of aflatoxigenic fungi in smoke-dried fish at marketing centers in the Jos metropolis. Total fungal load per sample was derived from plate counts and expressed as colony-forming units per gram of sample (cfu/g). *In-vitro* aflatoxigenicity of mould isolates was evaluated on coconut extract agar by exposing reverse side of plates to 365 nm ultra violet light. The results show that mean fungal load of smoke-dried fish ranged between  $2.00 \times 10^3 \pm 8.49 \times 10^2$  to  $3.09 \times 10^4 \pm 8.85 \times 10^3$  cfu/g. Generally, the processed fish was contaminated with combinations of eight fungal genera: *Fusarium*, *Aspergillus*, *Saccharomyces*, *Penicillium*, *Mucor*, *Rhodotorula*, *Schizosaccharomyces*, *Acremonium* and *Rhizopus*. Strains of *Penicillium digitatum*, *Fusarium equiseti* and *Fusarium semitectum* were the most predominant at 61.67%, 30.00% and 26.67% respectively. Comparatively, the assessment shows that smoke-dried fish from Terminus were the most contaminated ( $P < 0.05$ ) followed by samples from Chobe and Katakò markets. Out of 164 fungal isolates, only strains of *Aspergillus flavus* 5(8.33%) from Terminus market exhibited aflatoxin producing potential. In view of sea food safety and quality, the

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presence of toxigenic fungi on smoke-dried fish is of health significance and increase the risk of mycotoxin poison. The findings of this study call for stiff regulation and monitoring of smoke-dried fish in our open markets.

*Keywords: Aflatoxigenic; fungi; smoke-dried fish.*

## 1. INTRODUCTION

Fish constitutes a major source of animal protein and income for a vast majority of the population in Nigeria, particularly in riverine communities suited for fish cultivation. Incidentally, fish is one of the most perishable of all stable commodities and start to deteriorate as soon it leaves the water [1]. Generally, fish spoilage is accompanied by various physical and chemical changes, hence it must be subjected to some forms of processing as soon as captured [2]. In the tropical countries such as Nigeria, smoke drying of fish is one of the oldest available local forms of preservation methods essentially assumed by most fishing communities. This is practised because it is the form to obtain a product that can be stored conveniently at ambient conditions. Principally, smoke-drying of fish also impact antimicrobial effects that could enhance longer shelf life of the fish product [3].

Therefore, dry smoked fish constitutes an important part of fish distribution in Nigeria with a marketing trends predict an increase in consumer demands. It is evident that lack of stiff standardization and monitoring system towards smoke dried fish had invariably permitted poor handling practices. This has therefore resulted to gross exposure of fish and fish products to a wide range of microbiological and chemical contaminations [4]. In Plateau state for instance, smoke-dried fish is usually transported from neighboring states using poor handling and storage facilities. At the market stalls, retailers similarly exposed continuously the fish up to when they are purchased [5,6,4.]. Due to this, a number of studies had reported contamination of smoked dry fish up to levels higher than admitted norms in Nigeria [7,5,8], Egypt [9] and Ghana [10]. Contamination of smoke-dried fish with *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum*, *Penicillium chrysogenum*, *Rhizopus stolonifer* and *Mucor racemosus* is an important warning signal for human consumption [8]. Smoke-dried fish although possesses low water activity however, combination of environmental factors could predispose these fungal contaminants to food spoilage and aflatoxin production [11]. Due to these, assessment of

microbiological quality of the fish product should acquire importance in view of consumers' safety [12,13]. Therefore the need for continuous evaluation of the mycological status of smoked dried fish at retail markets has raised major concerns among researchers.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

A total of 60 smoke-dried fish were randomly purchased from hawkers and open markets in Jos metropolis. Successively, 20 samples were obtained each from Terminus, Chobe, and Katako markets during the rainy seasons at a monthly interval. The samples were then transported in clean polythene bags to the Department of Microbiology laboratory University of Jos for analysis.

### 2.2 Mycological Analysis

Mycological analysis of the samples collected was carried out following standard mycological procedures [4,8]. Stock solutions were prepared by homogenizing 25 g pulverised smoke-dried fish in 250 ml of sterile distilled water. A ten-fold serial dilution and 0.1 ml of the resultant solution was spread plated on Potato Dextrose Agar supplemented with 40 µg/ml chloramphenicol. Colonies formed were enumerated (cfu/g) after 4 to 7 days of incubation at 27±2°C. Morphologically distinct colonies were then sub cultured on fresh media to obtain pure isolates and further maintained at 4°C on Potato Dextrose Agar slants.

Identities of fungal isolates were certified based on their cultural, microscopic morphologies and comparison with confirmed representative of species in relevant fungal atlas [14,15].

### 2.3 Screening for Aflatoxigenic Fungi

Assessment of aflatoxin production potentials was performed on Coconut Dextrose Agar (CDA) following standard methods [16,17]. One hundred grams of shredded coconut was

homogenized for 5 min in 200 ml of hot distilled water. The homogenate was then filtered through a four layered-cheesecloth unit. The clear filtrate was adjusted to pH 7.0 using solution of 2NaOH. A quantity of agar (39 g/l) was then added and the mixture sterilized by autoclaving at 121°C for 15 mins. Chloramphenicol a broad spectrum antibiotic was added at 0.01 g per litre just before dispensing the medium into the Petri dishes to suppress bacterial contaminants. All pure fungal isolates were inoculated and incubated at 28°C for 5 days, using non inoculated coconut agar medium as control. The aflatoxin-producing potential of the isolates was determined by observing reverse side of plates under 365 nm ultra violet lamp. The emission of a characteristic blue fluorescence is a confirmation of the presence of aflatoxin producing potential of the isolates.

## 2.4 Data Analysis

The results of fungal loads and occurrence of fungi are presented as frequency distribution tables. Data sets were examined by one-way analysis of variance (ANOVA) using Statistical package for Social Sciences (SPSS) 95% level of significance using Minitab version 14 (Minitab Inc.).

## 3. RESULTS AND DISCUSSION

This study showed that smoke-dried fish samples were contaminated with fungal species that result in an essential total fungal load. Generally, the smoke-dried fish at retail outlets were contaminated with varying levels of fungal load of 0.00 to  $5.63 \times 10^4 \pm 4.00 \times 10^3$  cfu/g (Table 1). The result also showed that the smoke-dried fish obtained from Chobe market had the highest fungal load ( $3.09 \times 10^4 \pm 8.85 \times 10^3$  cfu/g), while samples from Katako markets had the least fungal count ( $2.00 \times 10^3 \pm 8.49 \times 10^2$  cfu/g). The study revealed that 75% of samples purchased from Terminus were contaminated, compared to 80% and 15% from Chobe and Katako correspondingly. Generally, *Schizosaccharomyces pombe* ( $5.63 \times 10^4 \pm 4.00 \times 10^3$  cfu/g) and *Saccharomyces cerevisiae* ( $2.10 \times 10^4 \pm 2.33 \times 10^3$  cfu/g) were accounted for highest fungal load on the dried products.

Fourteen fungal species were isolated from smoked dried fish as shown in Table 2. Out of 60 samples analyzed, 66.67% were contaminated with fungal species, with 56.67% of them being due to *Aspergillus* species. Other fungal genera

isolated from the fish product included *Mucor*, *Rhizopus* and *Fusarium*. Generally, *Penicillium digitatum* 37(61.67%) and *Fusarium equiseti* 18(30.00%) showed the highest incidence of major fungal pathogens. Apparently, the occurrence of fungal pathogens on fish samples in Terminus market 61(37.20) was significantly higher ( $P = 0.05$ ) than from Chobe (31.71%) and Katako (31.10%) outlets. However, there was significant ( $P = .05$ ) difference in the diversity of fungal species among the different fish markets.

The result showed that out of 34 *Aspergillus* sp. isolated, only 5 isolates belonging to strains of *A. flavus* exhibited aflatoxin-producing potential (Table 3). The incidence of aflatoxigenic *A. flavus* in the smoked dried fish from the markets was 8.33%. Detail result revealed that the aflatoxigenic *A. flavus* occurred only at 25.00% level in fish product from Terminus markets.

This study has shown that the smoke-dried fish sold in the markets in Jos metropolis are contaminated with fungal species at a significant total fungal load. This contamination level ( $1.69 \times 10^5 \pm 1.20 \times 10^4$ ) is however not substantially different from load of  $7.00 \times 10^3$  cfu/g previously reported by Olayemi and co workers [18] in Kano. Similarly in India, a contamination level of  $1.3 \times 10^4$  to  $2.2 \times 10^4$  cfu/g had been reported for sun-dried fish [19],  $1.1 \times 10^2$  to  $9.3 \times 10^4$  cfu/g in Ghana for smoke-dried fish [10] and 5.22 Log<sub>10</sub> (cfu/g) for smoke-dried fish in Cameroon [20]. Therefore, the quality of the smoke-dried fish seems to be a general reflection of the product in most of our dominant fish markets. This probably could essentially be linked to the adoption of similar traditional techniques of preparation, storage, handling and packaging of the fish product among both producers and vendors. And this could also be probably responsible for several reported cases of unsatisfactory microbial quality of smoke-dried fish at most markets centres [20,8]. Incidentally in Nigeria, many people routinely depend on this category of foods. Therefore the nonchalant attitude of government and people towards handling practices of foods sold in the open markets is a sign of their inability to prepare or even manage severe food outbreaks in future.

Similarly, the study showed that the contamination of the fish product is by a combination of fungi from more than one genus. These fungi are among the commonest fungi found to be associated with smoked fish samples in Nigeria; *A. flavus*, *A. terreus*, *A. fumigatus*,

Table 1. Fungal load of smoke-dried fish sold in Jos Metropolis

Fungi isolate	Total fungal load (cfu/g)/ number of isolates (n)			Mean count
	Terminus n = 20	Chobe n = 20	Katako n = 20	
<i>Fusarium equiseti</i>	2.00x10 <sup>3</sup> ±8.50x10 <sup>2</sup>	0.00	2.50x10 <sup>3</sup> ±1.06x10 <sup>3</sup>	1.25x10 <sup>3</sup> ±6.37x10 <sup>2</sup>
<i>Fusarium semitectum</i>	1.75x10 <sup>4</sup> ± 7.43x10 <sup>3</sup>	1.05x10 <sup>4</sup> ±4.46x10 <sup>3</sup>	0.00	9.33x10 <sup>3</sup> ±3.96x10 <sup>3</sup>
<i>Aspergillus niger</i>	1.00x10 <sup>3</sup> ±4.25x10 <sup>2</sup>	9.00x10 <sup>3</sup> ±3.82x10 <sup>3</sup>	0.00	3.33x10 <sup>3</sup> ±1.54x10 <sup>2</sup>
<i>Saccharomyces cerevisiae</i>	1.60x10 <sup>4</sup> ± 6.80x10 <sup>3</sup>	4.70x10 <sup>4</sup> ±1.99x10 <sup>4</sup>	0.00	2.10x10 <sup>4</sup> ± 2.33x10 <sup>3</sup>
<i>Penicillium expansum</i>	2.50x10 <sup>3</sup> ±3.61x10 <sup>2</sup>	0.00	0.00	8.33 x10 <sup>2</sup> ±1.20x10 <sup>2</sup>
<i>Mucor racemosus</i>	5.50x10 <sup>3</sup> ±2.34x10 <sup>3</sup>	5.00x10 <sup>3</sup> ±2.12x10 <sup>3</sup>	0.00	3.50x10 <sup>3</sup> ±1.49x10 <sup>3</sup>
<i>Rhodotorula mucilaginosa</i>	8.50x10 <sup>3</sup> ±2.34x10 <sup>3</sup>	5.15x10 <sup>4</sup> ±2.19x10 <sup>4</sup>	0.00	3.01x10 <sup>3</sup> ±8.53x10 <sup>2</sup>
<i>Schizosaccharomyces pombe</i>	0.00	1.69x10 <sup>5</sup> ±1.20x10 <sup>4</sup>	0.00	5.63x10 <sup>4</sup> ±4.00x10 <sup>3</sup>
<i>Acremonium butyric</i>	0.00	6.50x10 <sup>3</sup> ±2.76x10 <sup>3</sup>	0.00	2.17x10 <sup>3</sup> ±9.20x10 <sup>2</sup>
<i>Rhizopus oryzae</i>	1.30x10 <sup>3</sup> ±1.25x10 <sup>3</sup>	0.00	1.00x10 <sup>3</sup> ±4.25x10 <sup>2</sup>	7.67x10 <sup>2</sup> ±5.58x10 <sup>2</sup>
<i>Aspergillus oryzae</i>	0.00	4.00x10 <sup>3</sup> ±1.70x10 <sup>3</sup>	0.00	1.33x10 <sup>3</sup> ±5.67x10 <sup>2</sup>
<i>Aspergillus terreus</i>	0.00	2.50x10 <sup>3</sup> ±1.06x10 <sup>3</sup>	0.00	8.33x10 <sup>2</sup> ±3.53x10 <sup>2</sup>
<i>Aspergillus flavus</i>	5.00x10 <sup>3</sup> ±2.12x10 <sup>3</sup>	0.00	0.00	1.67x10 <sup>3</sup> ±7.07x10 <sup>3</sup>
<i>Penicillium digitatum</i>	0.00	1.27x10 <sup>5</sup> ±5.42x10 <sup>4</sup>	2.45x10 <sup>4</sup> ±1.04 x10 <sup>4</sup>	8.59x10 <sup>3</sup> ±2.15x10 <sup>4</sup>
Total mean	4.24x10 <sup>3</sup> ±1.71x10 <sup>3</sup>	3.09x10 <sup>4</sup> ±8.85x10 <sup>3</sup>	2.00 x 10 <sup>3</sup> ±8.49 x10 <sup>2</sup>	

n = number of samples; 0.00 = no growth

*A. niger*, *Mucor* sp., *Cladosporium* sp., *Penicillium* sp., *Candida tropicalis* and *Fusarium moniliformis* [21,5,22]. In view of sea food safety, the presence of fungal species such as *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. and *Fusarium* sp. in food are of medical implication. Majority of these species have been reported to be pathogenic [23]. Similarly, *Aspergillus* species such as *A. flavus*, *A. parasiticus* and *A. nomius* are the most notorious of the common isolates. This is due to their high potentials for producing aflatoxin and other mycotoxins in line with the findings of this study [24].

A very glaring finding of this study affirmed variation in contamination level of the fish product with market locations [10,9,8]. Apart from handling practices, this finding proposes that environmental factors could also be playing dominant role. Comparatively, the fish samples from Terminus market were the most contaminated ( $P < 0.05$ ) followed by samples from Chobe and Katako markets. Apparently, the hawking activity that characterised smoked dried fish vendors around Terminus market could have predisposed the fish products to conditions that influenced fungal development. Consequently, this might be responsible for the gross variation in occurrence of fungal species observed among vendors as well as increased susceptibility to toxin production. Comparatively, most traders in

Chobe and Katako had their foodstuffs stocked in cubicles and this will definitely prevent excessive exposure of products to contaminations. This similarly affirmed the worries of Oyebamiji and Oyebimpe [8] on the handling practices and unhygienic environments in which retailers often display smoked-dried fish. Accordingly, this suggests the need for critical approaches to protect exposed food condiments and food stuffs through good packaging and handling practices.

Although moulds may be present in foods as contaminants, nevertheless the presence of toxigenic species increases the risk for mycotoxin production [25]. From this study it may be appropriate to suggest that susceptibility of smoke-dried fish to contamination by aflatoxigenic strains is low compared to other foods/ crops such as maize from Nigeria [26, 27]. Among the moulds isolated, only strains of *A. flavus* from Terminus market presented aflatoxigenic producing potentials. Importantly, this study is consistent with the fact that fungi can proliferate in the tissue of the fish product. Therefore the present mycological status of smoke-dried fish at the retail markets sampled in Jos metropolis call for concerted efforts by relevant regulatory agencies to develop stringent food regulations and control strategies and publicise these through effective safety campaigns.

**Table 2. Occurrence of fungi on smoke-dried fish sold in some markets in Jos Metropolis**

Fungi isolate	Occurrence of Fungi (%) / Number of Isolates			
	Terminus n = 20	Chobe n = 20	Katako n = 20	Total
<i>Fusarium equiseti</i>	6(30.00)	8(40.00)	4(20.00)	18(30.00)
<i>Fusarium semitectum</i>	12(60.00)	0(0.00)	4(20.00)	16(26.67)
<i>Aspergillus niger</i>	7(35.00)	6(30.00)	0(0.00)	13(21.67)
<i>Saccharomyces cerevisiae</i>	8(40.00)	6(30.0)	0(0.00)	14(23.33)
<i>Penicillium expansum</i>	5(25.00)	0(0.00)	0(0.00)	5(8.33)
<i>Mucor racemosus</i>	3(15.00)	0(0.00)	2(10.00)	5(8.33)
<i>Rhodotorula mucilaginosa</i>	7(35.00)	8(40.00)	0(0.00)	15(25.00)
<i>Schizosaccharomyces pombe</i>	0(0.00)	4(20.00)	0(0.00)	4(6.67)
<i>Acremonium butyric</i>	1(5.00)	3(15.00)	0(0.00)	4(6.67)
<i>Rhizopus oryzae</i>	5(25.00)	1(5.00)	6(30.00)	12(20.00)
<i>Aspergillus oryzae</i>	0(0.00)	0(0.00)	11(55.00)	11(18.33)
<i>Aspergillus terreus</i>	0(0.00)	0(0.00)	4(20.00)	4(6.67)
<i>Aspergillus flavus</i>	6(30.00)	0(0.00)	0(0.00)	6(10.00)
<i>Penicillium digitatum</i>	1(5.00)	16(80.00)	20(100.00)	37(61.67)
Total	61(37.20)	52(31.71)	51(31.10)	164(100.00)

n= number of samples

**Table 3. Occurrence of aflatoxigenic fungi on smoked dried fish sold in Jos Metropolis**

Fungi isolate	Number of positive isolates/occurrence of aflatoxigenic fungi (%)			
	Terminus n = 20	Chobe n = 20	Katako n = 20	Total
<i>Fusarium equiseti</i>	-	-	-	-
<i>Fusarium semitectum</i>	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-
<i>Saccharomyces cerevisiae</i>	-	-	-	-
<i>Penicillium expansum</i>	-	-	-	-
<i>Mucor racemosus</i>	-	-	-	-
<i>Rhodotorula mucilaginosa</i>	-	-	-	-
<i>Schizosaccharomyces pombe</i>	-	-	-	-
<i>Acremonium butyric</i>	-	-	-	-
<i>Rhizopus oryzae</i>	-	-	-	-
<i>Aspergillus oryzae</i>	-	-	-	-
<i>Aspergillus terreus</i>	-	-	-	-
<i>Aspergillus flavus</i>	5(25.00)	-	-	5(25.00)
<i>Penicillium digitatum</i>	-	-	-	-
Total	5(25.00)	0(0.00)	0(0.00)	5(8.33)

(-) = not aflatoxigenic

#### 4. CONCLUSION

The smoke-dried fish at retail outlets were contaminated with varying levels of fungal load of  $5.63 \times 10^4 \pm 4.00 \times 10^3$  cfu/g. Predominantly, out of fourteen fungal species isolated, 56.67% of them is due to *Aspergillus* species however, *Penicillium digitatum* and *Fusarium equiseti* showed the highest incidence of major fungal pathogens. This study therefore showed susceptibility of smoke-dried fish to contamination by aflatoxigenic strains of *A. flavus*.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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