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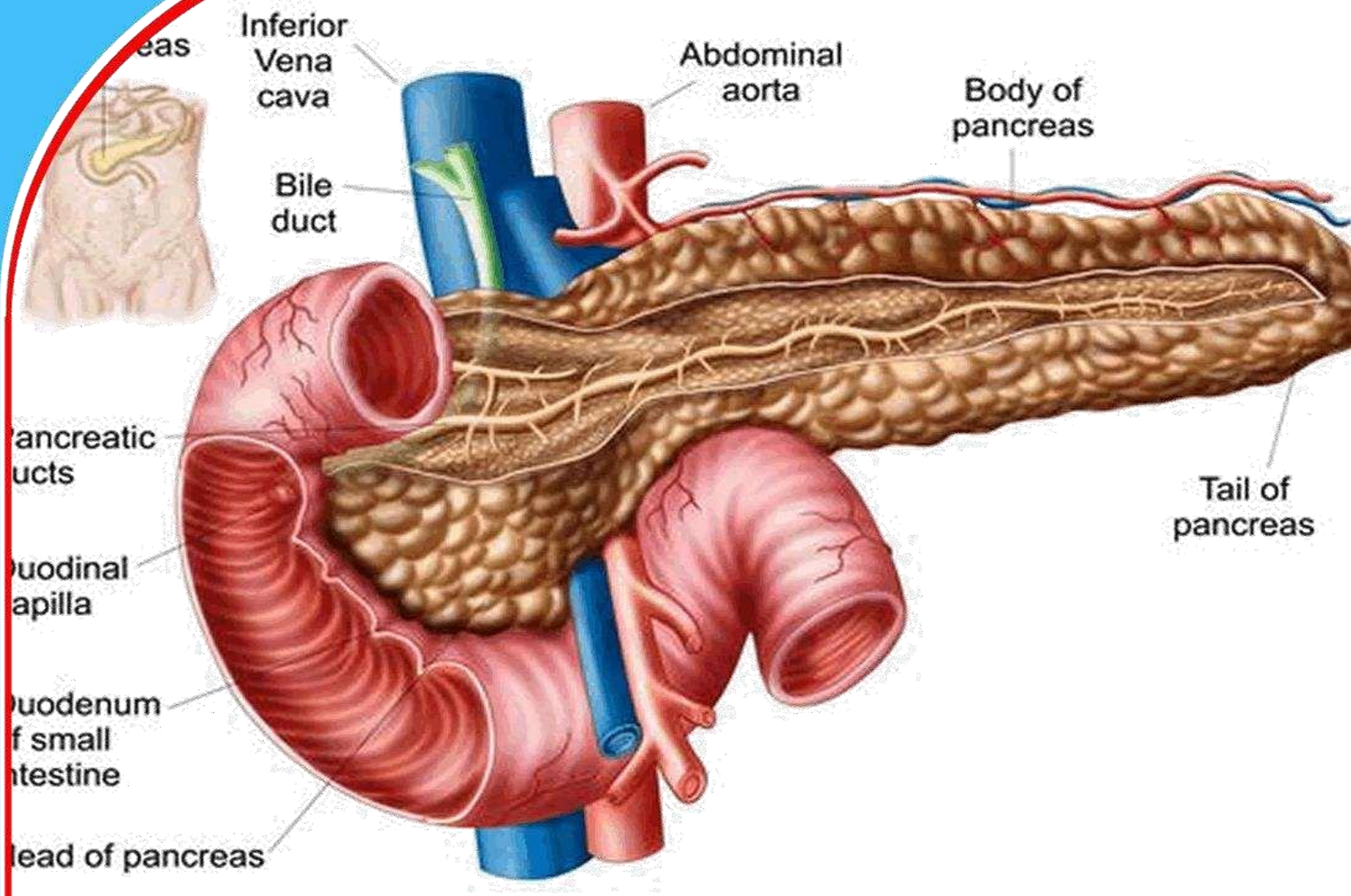

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MYCOLOGICAL EXAMINATION OF SMOKE-DRIED FISH SOLD IN BIDA MAJOR MARKETS

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ABSTRACT

Purpose: This research was conducted to determine the occurrence of aflatoxin producing fungi in smoke-dried fish in Bida.

Methodology: The study was carried out at the Microbiology Laboratory Federal Polytechnic, Bida, Niger state. Smoke dried fish samples were collected randomly from three major markets in Bida town. Fungi isolation was done after serial dilution using Potato dextrose agar. Total fungal load per sample was obtained from plate counts and expressed as colony-forming units per gram (cfu/g). The isolates were identified microscopically using Lactophenol cotton blue stain. Aflatoxigenicity text was done using coconut extract agar and exposed to 365 nm ultra violet light. Data collected were analysed by one-way analysis of variance (ANOVA) at $P < 0.05$.

Findings: The results showed maximum mean fungal load of smoke-dried fish of$9.54x10^5 \pm 1.83x10^6$ cfu/g. Fungi associated with the smoked dried fish belong to five genera: *Aspergillus, Penicillium, Candida, Acremonium* and *Rhizopus*. Comparatively, the assessment showed that smoke-dried fish from old market were the most contaminated followed by samples from small market and modern markets. *Aspergillus flavus* had the highest prevalence of 32.88 %. Only strains of *Aspergillus flavus* gave positive to aflatoxins, out of the 24 *Aspergillus flavus* studied, only 25 % were positive to aflatoxins. Old market exhibited the highest of aflatoxin producing fungi.

Unique contribution to theory, practice and policy: In view of this results, there is need to adopt hygienic practices during smoke dried fish processing and storage in Bida to avoid increase risk of aflatoxin poisoning.

Keywords: *Fish, Fungi, Aflatoxin, Aspergillus flavus, Bida*

Introduction

In Nigeria and the world at large, fish is a major source of animal protein and it serves as income for a vast majority of the population in the world, particularly riverine dwellers. Fish has shown to be a perishable stable food with high level of deterioration immediately outside water bodies (Ames et al., 2014). Deterioration of fish occurs as a result of complex enzymatic, microbial, chemical and physical changes (Junaid et al., 2010; Abba, 2012). In Nigeria, fish preservation is done to prevent spoilage during storage and taste addition. One of the traditional means of fresh fish preservation in Nigeria is by smoke drying (Job et al., 2016).

Fungal contamination is a serious challenge faced by fish farmers and consumers in Africa. This infection may be caused by fungi as the initial deterioration agents or as a secondary contaminant due to mechanical damage (Eyo, 2012). Several moulds has being implicated in fish spoilage, which is also harmful to consumer's health because of their ability to produce mycotoxins. Fungal fish spoilage has also resulted in food insecurity, shortage of essential nutrients and low income generation in developing countries (Wu, 2014).

Mycotoxins, especially aflatoxins are major contaminant of feed worldwide (Barbosa et al., 2013; Fallah et al., 2014). *Aspergillus* species are the most implicated fungal species that produces Aflatoxins, aflatoxins are secondary metabolites which are highly toxic, carcinogenic and Mutagenic (Khlangwiset et al., 2011).

Lack of adequate standardization of smoked dried fish had led to unhygienic practices causing exposure of fish and fish products to various forms of contaminations (Wogu. 2011) . Smoked dried fish in Niger state is so ld, transported and stored under poor condition. This necessitated the need for examination of mycological states of smoked dry fish sold in Bida. Job et al. (2016) reported presence of aflatoxins produced by *Aspergillus* species among other fungi species isolated from smoked dry fish in Jos. However, little information is available on the mycotoxin production in dried fishes of Bida, Niger state; hence this study is aimed at evaluating the aflatoxin presence in dried fishes sold in major markets in Bida.

Materials and Methods Sample Collection

A total of 9 smoke-dried fish were randomly purchased in Bida major markets. Three each from modern market, old market and small market. The samples were collected at different locations within each market and at monthly interval. The samples were then transported aseptically in a clean polythene bags to the Department of Microbiology laboratory, Federal Polytechnic Bida, Niger state for further examination.

Fungi Isolation and Identification

Stock sample of the collected smoked dried fish samples were prepared by homogenizing 25

g of pulverized smoke-dried fish in 250 ml of sterile distilled water. A ten-fold serial dilution was carried out and 0.1 ml of the solution spread on plated Dextrose Agar, amended with 40 g/ml chloramphenicol. Colonies formed were counted after 3-5 days incubation in a Laboratory locker. Morphologically distinct colonies were then subcultured on fresh media to obtain pure isolates. The isolated fungi were identified based on their cultural, microscopic morphologies and comparison wit h confirmed representative of species in relevant fungal atlas.

To identify the fungi, a method described by John et al. (2018) was adopted. A drop of lactophenol cotton blue stain was placed on a clean grease-free glass slide. A small fragment of colony, woolly or powdery colony was picked at mid-point of culture using a sterile needle and teased in the stain unt il a homogenous blue mixture of stain and culture wad obtained. A clean cover slip was applied carefully to avoid air bubbles and examined using x 10 and x 40 objective of the microscope respectively.

Screening for Aflatoxigenic Fungi

Assessment of aflatoxin production potentials was performed on Coconut Dextrose Agar (CDA) following method describe by Job et al. (2016). 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 and HCl and NaOH. Agar (39 g/L) was then added and the mixture sterilized by autoclaving at 121 °C for 15 Mins. Chloramphenicol 0.01g per liter was introduced before dispensing the medium into the Petri dishes. Pure fungal isolates were aseptically inoculated and incubated at for 5 days in a Laboratory locker, non-inoculated coconut agar medium served as control. The aflatoxin-producing potential of the isolates were determined by observing reverse side of plates under 365 nm ultra violet lamp. The emission of a characteristic blue florescence indicated the presence of aflatoxin producing potential.

Data Analysis

Data sets were examined by one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) 95% of significance using Minitab version 14 (Minitab Inc.). Results are presented in tables.

Results and discussion

The result showed on Table 1 showed the fungi load of smoked dried fish sold in Bida markets. The result indicated the various markets were contaminated with varying load of fungi ranging from $5.44x10^5 \pm 1.21x10^6$ to $9.54x10^5 \pm 1.83x10^{6.}$. The results on the Table 1 also demonstrated that Smoked dried fish collected from Small Market had more fungi load compared to others. The fungi load of the smoke dried fish from old market, small market had the highest fungi load, while sample from the modern market had the least fungi load. This worked indicated that smoke dried fish in three major markets in Bida town are contaminated with a total fungal load of $6.63x10^6 \pm 1x21x10^7$. This level of infestation is similar with work of Nyarko et al. (2011) which

indicated fungal load of smoke dried fish of 9.3x104 cfu/g in Ghana. Similar work done in Kano by Olayemi et al. (2012) reported a fungal load of 7.00x103 cfu/g. This high level of contamination may be associated with unhygienic practices by processors and sellers. According to Wogu (2011), lack of adequate standardization of smoked dried fish had led to unhygienic practices causing exposure of fish and fish products to various forms of contaminations. Apart from handling practices, this finding proposes that environmental factors could also be playing dominant role.

Comparatively, the fish samples from old and small market were the most contaminated followed by samples from and modern markets. Apparently, the hawking activity that characterized smoked dried fish vendors around old market could have predisposed the fish products to conditions that influenced fungal development. Oyebamiji and Oyebimpe (2013) reported that the unhealthy environments in which sellers often display smoked-dried fish contribute significantly to the contamination of smoke dried fish.

Table 1: Total Viable Count

Sample Location	Sampling points	Fungi count (CFU/g)
SM	A1	$7.12x\ 10^{5}\pm1.30x10^{6c}$
	A2	$9.16x10^{5} \pm 1.74x10^{6a}$
	A3	$8.11x10^{5} \pm1.52x10^{6b}$
OM	A1	$6.94x10^{5}\pm1.30x10^{6d}$
	A2	$9.54x10^{5}\pm1.83x10^{6a}$
	A3	$6.31x10^{5\pm}1.22x10^{6d}$
MM	A1	$6.80x10^{5}\pm1.30x10^{6d}$
	A2	$5.44x10^{5}\pm1.21x10^{6e}$
	A3	$6.94x10^{5}\pm1.30x10^{6d}$
Total	(AI,A2,A3)	$6.63x10^{6}\pm1x21x10^{7}$

Each value is the mean ± standard deviation of five (5) determinations. Similar alphabets with each column are not significantly different (P>0.05) A l= is first sampling point, A2 = is second sampling point, A3- is the third sampling point from three (3) different location.

KEY: SM = Small market, OM= Old market, MM- Modern market.

The results on Table 2 showed the various fungi species isolated from the smoked dried fish samples from the three major market in Bida town. Mycobiota of smoke dried fish samples the various markets studied in this work revealed a total of 8 species belonging to 5 genera such as Aspergillus, Rhizopus, Penicillum Candida albican and Acremonium butyric. These fungi are among the commonest fungi implicated with deterioration of smoked fish samples in Nigeria. This

finding is in agreement with the earlier work of Wogu and Maduakor (2010) and Fredrick et al. (2015) who reported that these fungi were they major causes of microbial spoilage of fresh fish after capture. Similar studies carried out by Olayemi et al. (2012) in Kano and Job et al. (2016) in Jos reported the same species of fungi. Most of these fungal species have been found to be pathogenic (John et al., 2016). *Aspergillus* species being the most notorious among all the common isolates associated with smoke dried fish

The results in Table 3 revealed frequency distribution of the fungi isolates. Generally, the fungal species varied from one market to another. *Aspergillus flavus* revealed the most prevalent species isolated from all the smoke dried fish samples, with occurrence of 32.88% while *Rhizopus oryzac* and *Acremonium butyric* gave the minimum occurrence of 1.37 %. This present result were similar to the work of Atef et al. (2011) who report 50 %
Aspergillus flavus occurrence compared to other fungal species. Ibrahim (2000) and Nasser (2002) also showed presence of *A. flavus* from most of all samples of fish examined in their studies. Fish are more liable to contamination with *A. flavus* from animal and human reservoirs which may contaminate the water in the fishing area. Furthermore, contamination during handling and processing may occur, with increased contamination associated with fish caught from polluted areas (Hassan et al., 2007).

Table 2 Cultural and microscopic characteristic of fungi isolate from smoked dried fish

Cultural Characteristic	Microscopic examination	Probable fungi isolates
The isolate surface was powdery, showing shades of Green with a Narrow white border.	Hyphae were septate with smooth walled conidiophores.	*Aspergillus fumigatus*
The colonies showed shades of green and some white.	Conidiophores were hyaline, and rough walled. Conidia appeared long dry chains and columns.	*Penicllium expansum*
Powdery black colonies with white periphery	Non septate hyphae, large spores head with conidia in chains.	*Aspergillus niger*
Fast growing colonies with white cotton becoming grey to blackish grey	Smooth walled, non-septate which sporangia are globose.	*Rhizopus oryzae*
Slow growing, compact and moist colonies. At first powdery,and floccose with age. White, and pinkish in colour.	The conidia are one celled. Pigmented, globose to cylindrical and mostly aggregated	*Acremonium butyric*
Colonies were typically suede-like. Brown in colour with a yellow to deep dirty brown reverse	Conidial heads are compact, conidiophores stripes were hyaline and smooth-walled	*Aspergillus butyric*

Aspergillus flavus
Colonies are granular, flat, They have radiating conidia often with radial grooves. heads while the
Yellow At first but quickly conidiophores appear rough

becoming bright to dark yellow
green with age

White dot colonies with moist Spherical to sub spherical *Candida albicans* smooth surface and white to budding blastoconidia cream coloured

Table 3: Occurrence of fungi on smoked dried fish sold in Bida Markets.

Fungi Isolates	SM	OM	MM	Total
Aspergillus fumigate	4(11.11)	2(10.00)	2(11.76)	8(10.96)
Penicillum expansum	4(11.11)	2(10.00)	5(29.41)	11(15.07)
Aspergillus niger	6(16.67)	4(20.00)	4(23.53)	14(19.18)
Rhizopus oryzae	0(0.00)	1(5.00)	0(0.00)	1(1.37)
Acremonium butyric	1(2.78)	0(0.00)	0(0.00)	1(1.37)
Aspergillus terreus	10(27.76)	2(10.00)	0(0.00)	12(16.44)

Aspergillus flavus	11(30.56)	7(35.00)	6(35.29)	24(32.88)
Candida albican	0(0.00)	2(10.00)	0(0.00)	2(2.74)
Total	36(49.32)	20(27.40)	17(23.29)	73(100.00)

KEY: SM = Small market, OM= Old market, MM- Modern market.

Table 4 showed the presence of aflatoxin associated with smoked dried fish samples collected. The result revealed that only *Aspergillus flavus* produced aflatoxin. The highest presence of aflatoxin was detected in smoke dried fish sampls from Old market with Small Market shown the least. Among the 7 *Aspergillus flavus* obtained from Old Market, 3 (42.68 %) indicated aflatoxin presence while only 1(9.09 %) out of the 11 *Aspergillus flavus* obtained from Small Market indicated presence of aflatoxin. The Aflatoxin occurrence in smoke dried fish was found to be higher (42.86 %) than that reported by Job et al. (2016), who detected 33.33% aflatoxins presence in smoke dried fish from major markets in Jos metropolis. This could be attributed to seasonal variations and hygienic practices by processors and retailers in the two different locations.

Fish are usually exposed to smoking and direct sunlight for drying and the dry fish are maintained at the ambient temperature for storage and sale in Nigeria. Normally, the smoke dried fish are kept in retail outlets for months in ambient temperature without packing. The low water activity of the product together with the high ambient temperatures creates favourable environment for potential proliferation of many toxigenic fungi in dried food commodities (Fredrick et al., 2015). Depending on the exposure, contamination of fish with aflatoxins can induce adverse health effects such as poor growth rates and presence of gross and microscopic lesions in fish. These lead to economic losses due to low production, morbidities, mortalities and poor quality of fish and fish products (Mahfouz and Sherif, 2015).

Table 4: Occurrence of Aflatoxins

Fungi Isolates	SM	OM	MM	Total
Aspergillus fumigate	-	-	-	-
Penicillum expansum	-	-	-	-
Aspergillus niger	-	-	-	-
Rhizopus oryzae	-	-	-	-
Acremonium butyric	-	-	-	-
Aspergillus terreus	-	-	-	-
Aspergillus flavus	1(9.09)	3(42.86)	2(33.33)	24(25.00)
Candida albican	-	-	-	-

KEY: - = Absence of aflatoxin, SM = Small market, OM= Old market, MM- Modern market.

Conclusion

The results from this studied demonstrate varying levels (2.74 - 32.88%) of fungal contamination of smoke-dried fish sold in major Bida markets with fungi species. *Aspergillus flavus* gave the highest prevalence when compared to other fungal isolates. The result obtained in this work also showed Old Market had the highest (42.86 %) presence of aflatoxins indicating the susceptibility of smoked dried fish sold in Bida to contamination. This study is the confirmation of infection and contamination of smoked dried fishes with mycotoxin producing fungi and other fungal species in major markets in Bida, Niger state.

Recommendations

Further research is require to extract and characterized the aflatoxin produced by the fungal species isolated in this study and also to determine the relationship between storage condition of smoked dried fish and presence of aflatoxin. Therefore, with the results evidence in study, proper handling of smoke dried fish should be employed. This becomes necessary as the consumption of aflatoxin can lead to serious health hazard to humans who are the final consumer of the smoked dried fish products.

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