FUNGAL TERRITORY
2021, VOL. 4, NO. 2, 9-11

OPEN ACCESS
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REGULAR ARTICLE

INCIDENCE OF MYCOTOXINS IN MOULDY SMOKED DRIED FISH AND MEAT (KUNDI) MARKETED IN SOUTHWESTERN NIGERIA

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https://doi.org/10.36547/ft.300

ABSTRACT

The mycotoxicological safety of 85 mouldy dried fish (n=40) and meat (n=45) samples purchased from Ijebu-ode, Ogun State and Aleshinloye, Ibadan, Oyo State markets both in South Western part of Nigeria respectively, was assessed due to heavy consumption of these animal protein sources by many low-income families in Nigeria. The presence of aflatoxigenic moulds and levels of aflatoxins in the samples were determined by the dilution plating technique and high performance thin-layer chromatographic method. The predominant fungal species isolated from the samples was *Aspergillus niger*-clade (49.65%); other species isolated include *Aspergillus* section *Flavi* (36.83%) and (13.52%). All samples analysed for mycotoxin presence were contaminated with aflatoxin B_1. Aflatoxin B_2, G_1 and G_2 were below detectable limits. About 13% of the samples had aflatoxin B_1 concentration higher than the maximum acceptable level (10 ng/g). Results imply that the consumption of mouldy dried meat can result in serious public health hazard and hence there is need for advocacy programs to enlighten the populace on proper processing and storage of meat products.

Keywords: Aflatoxin, Contamination, Food safety, Fungal

INTRODUCTION

Food safety and security remain a major concern in the sub-saharan Africa **(Bankole and Adebanjo, 2003)**, several food contaminants threaten the integrity of food products and make food unfit for consumption. Amongst the several food contaminants, mycotoxin in food commodities remain a challenge. In Nigeria, meat and fish consumption is usually associated with individual's socio-economic status, meat has been processed and eaten in various form that include *suya*, *kilishi* and *tinko* (**Obanu *et al*., 1987**). Dried meat also known as *kundi*, *tinko* and *banda* in Yoruba and Hausa respectively is a cheap, poor quality meat derived from various types of livestock (donkeys, horses, camel and buffalo) (**Adeyeye, 2016**). It is usually salted and spiced with condiments like ginger and garlic before being subjected to drying. When drying by sun or wind, the potential source of polycyclic aromatic hydrocarbons is the environment (**Ekhator et al, 2018**). Contamination can originate from soil/dust or/and from combustion from industry and from traffic as well as forest fires and volcanic eruptions (**Manisalidis et al, 2020**). This form of processed meat is included in diet because meat is a nutritious food that contains quantities of essential amino acids in forms of protein, it contains B group vitamins especially niacins and riboflavin, iron, phosphorus, ash and calcium. According to **Omotosho (2004)**, the recommended daily minimum protein required by an adult in Nigeria is between 65 and 85g per person and 35g of this minimum requirement should be obtained from animal products.

Chitrakar et al. (2019) reported that dried food including fish and meat are not inherently safe because of food borne illnesses caused by consumption of dried foods contaminated with *Salmonella* spp., *Cronobacter* spp., *Staphylococcus* spp. and *E. coli*. **Adesokan et al. (2016)** opined that the major source of contaminants especially aflatoxins was as a result of poor handling, packaging and storage of fish and meat alike. The processing technique involved in the processing of Kundi and dried fish; storage conditions and transportation mode predispose the meat product to mould growth and mycotoxin production. Mycotoxins are secondary metabolites produced by fungi in various food commodities and feed (**Adeyeye, 2016; Ezekiel et al., 2012; Atanda *et al*., 2013**). Out of the 300 secondary metabolites produced by fungi, about 30 metabolites have been found to be harmful and toxic among which Aflatoxins are a group of mycotoxins produced by *Aspergillus* subgenus section *Flavi* particularly *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergilus cerealis* (**Ezekiel *et al*., 2014**). Aflatoxins are grouped into namely AFB_1, AFB_2, AFG_1, AFG_2. AFB_1 is considered to be the most potent metabolite, it has been classified under class 1 human carcinogen by International Agency for Research on Cancer (IARC), it is highly hepatoxic, mutagenic and genotoxic (**Seo et al., 2011; IARC. 2012**).

Considering the detrimental effects of aflatoxins in the human system, there is need to assess and quantify the metabolites in staple and essential food products to ensure food safety. This study therefore aimed at quantifying the level of aflatoxins in dried fish and meat sold in Ogun and Oyo states of Nigeria.

MATERIALS AND METHODS

Sampling

Samples of smoked dried fish (n = 40) and smoked dried meat (n = 45) were purchased from vendors in markets in Ogun and Oyo states of Nigeria, respectively. The markets in both states were purposively selected because they are major depot for both commodities. Only vendors who belonged to the commodity association and had at least 1–2 kg food sample in store were selected. A total of 10–15 vendors per food category were selected among those who met the aforementioned criteria for sample collection. Each vendor was interviewed in order to retrieve information on the source, storage conditions and duration as well as extent of consumption of the foodstuffs.

Each sample (approximately 100 g) comprising of 5–10 visibly moldy pieces of either smoked dried fish or meat were collected by simple randomization from a vendor's tray/basket in the open market. Fish samples were purchased once every two weeks for two months while meat samples were obtained once every month for three months. Each sample was comminuted, quartered to yield about 25 g sub-sample and kept at -20ºC until further analysis.

Mycological analysis of food samples

Isolation of moulds

The dilution plating technique of **Samson *et al*. (1995)** was employed to assess the mycological profile of the dried fish and meat samples. Briefly, 10g of the ground samples were suspended in 90ml of sterile distilled water, homogenized for 2 minutes and spread-plated on potato dextrose agar (PDA) supplemented with 0.02% chloramphenicol and streptomycin. All isolations were performed in triplicates and the inoculated plates were incubated for 3–5 days at 31ºC. All colonies were counted and fungal load expressed as Log_{10} colony forming units per gram (log_{10}CFU/g) of sample analyzed. Colonies of *Aspergillus* were purified on PDA and transferred onto neutral red desiccated coconut agar (NRDCA) for further characterization as described by **Ezekiel *et al*. (2014)**.

Characterization of isolated moulds

The isolates on NRDCA were examined for their taxonomic confirmation. The species of *Aspergillus* was identified by assessing the morphology and for mycotoxin characterization, the aflatoxin-producing potential was determined. After five days of incubating the inoculated NRDCA plates at 31ºC, the macro

characters of species were assessed and compared with taxonomic descriptions in literature **(Samson *et al.*, 2002; Şesan *et al.*, 2007)**. To determine the isolates that produce aflatoxins, each isolate was centrally inoculated on freshly prepared plates and incubated in the dark at 30°C for 3 to 5 days. Each plate was checked under UV light (365nm wavelength) at 24 hours interval for fluorescence resulting from aflatoxin liberation and color fluorescence to determine the type of aflatoxin as previously described by **Ezekiel et al. (2014)**.

Aflatoxin determination in dried fish and meat samples

The fish and meat samples were analyzed for the presence of aflatoxins [aflatoxin B_1 (AFB_1), AFB_2, AFG_1 and AFG_2] by High Performance Thin Layer Chromatography (HPTLC) at the Pathology and Mycotoxin Laboratory of the International Institute of Tropical Agriculture, Ibadan, Nigeria. About 20 g of each sub-sample was extracted with 100 ml of 80% methanol by high-speed blending for 2 minutes and subsequent shaking for 30 minutes on an orbital shaker. The mixture passed through Whatman filter paper No. 1 and the filtrate was partitioned in a 250 ml separatory funnel to which 20 ml of 10% sodium chloride and 25 ml of hexane were pre-added. The methanol layer was collected into a 250 ml separatory funnel, mixed with 35 ml dichloromethane, shaken for 30 seconds and allowed to stand for separation. The lower dichloromethane layer was collected into a polypropylene cup and evaporated to dryness in a fume hood. The residue was re-dissolved in 1 ml of dichloromethane prior to aflatoxin quantification. Aflatoxin standards and sample extracts were separated on TLC plates (silica gel 60, 250 µm) in isopropanol-methanol-water (96:3:1, v/v/v). The plates were visualized under ultraviolet light and scored visually for the presence or absence of aflatoxin with a 2 ng/g limit of detection. Quantification was performed by the scanning densitometer as previously described (**Suhagia *et al.*, 2006**).

Data analysis

The SPSS® v. 16.0 was used for data analysis. Simple descriptive statistics was performed for aflatoxin distribution.

RESULTS

In each of the three different months of sample collection as shown in Table 1, fifteen samples were obtained and analyzed for fungal contamination. In the first month, thirteen of the samples were contaminated. In the second month, six of the samples were contaminated. In the third month, all fifteen samples were contaminated. Three specie of fungi were observed from the isolates.

Table 1: Frequency and Percentage of Fungal Isolates

		FREQUENCY	PERCENTAGE (%)
MONTH 1	*Aspergillus* section *Flavi*	48	48.98
	Aspergillus niger – *clade*	30	30.61
	Rhizopus spp.	20	20.41
MONTH 2	*Aspergillus* section *Flavi*	37	56.92
	Aspergillus niger – *clade*	25	38.46
	Rhizopus spp.	3	4.62
MONTH 3	*Aspergillus* section *Flavi*	73	27.44
	Aspergillus niger – *clade*	158	40
	Rhizopus spp.	35	13.16

Tables 2 and 3 show the range of Aflatoxin B_1 found in both dried meat and fish obtained in the markets. The results showed that the overall mean of AFB_1 detected in the meat samples and fish samples were more than 4µg/kg and 2µg/kg respectively.

Table 2: Distribution of Aflatoxin B_1 in Fish Sample

			Aflatoxin B_1(ng/g)	Concentration
Weeks	**Ns**	**Nc**	**Range**	**$\bar{X}$ ± SD (ng/g)**
1	10	2	0.00-8.11	2.92±3.85
2	10	4	5.05-7.94	5.97±1.33
3	10	2	0.00-5.93	2.75±3.19
4	10	3	4.87-6.59	5.71±2.88
Total	**40**	**11**	**0.00.8.11**	**4.25±2.88**

Legend: Ns – number of samples obtained, Nc –number of samples contaminated, $\bar{X}$ – mean, SD – standard deviation

Table 3: Occurrence of Aflatoxin B_1 in Dried Meat

				Aflatoxin B_1 concentration (ng/g)		
	Ns	**Na**	**Nc**	**RANGE**	**$\bar{X}$**	**S.D**
MONTH 1	15	5	5	3.10 – 6.93	4.66	1.52
MONTH 2	15	5	5	1.92 – 5.09	3.49	1.24
MONTH 3	15	5	5	2.60 – 18.94	8.53	6.82
ALL SAMPLES	45	15	15	1.92 – 18.94	5.56	4.40

Legend: Ns – number of samples obtained, Na – number of samples analyzed, Nc –number of samples contaminated, $\bar{X}$ – mean, SD – standard deviation

DISCUSSION

The results showed that the overall mean of AFB_1 detected in the meat samples and fish samples exceeded the stringent regulation of 4 µg/kg and 2 µg/kg respectively, set by the EU and this poses a great threat to the consumer's health because AFB_1 is a potent human carcinogen **(IARC, 2002)** that has been linked to hepatocellular carcinoma.

This was also in agreement with the findings of **Adesokan *et al*. (2016)** who investigated the aflatoxin concentration of smoked dried fish collected from Ibadan. In contrast to the aflatoxin levels in smoked dried fish sold in different markets in Abeokuta by **Akinyemi *et al*. (2011)**, the AFB_1 detected in the fish samples exceeded the results obtained from the samples collected from Abeokuta. Similarly, **Adebayo-Tayo *et al*. (2008)** reported high concentration of AFB_1 in smoked fish from Uyo, Nigeria. Human exposure by consumption of AFB_1 contaminated food can increase the chances of consumers developing hepatocellular carcinoma and stunted growth in children **(Turner *et al*., 2012)**. Considering the fact that dried fish and meat are highly consumed by the low-income populace of Nigeria, long term exposure can increase the occurrence of liver cancer. The findings from this study further substantiated the claims of **Chitrakar *et al*. (2019)** who concluded that dried food items especially fish and meat should be consumed with caution as they are not inherently safe.

CONCLUSION AND RECOMMENDATION

This study confirmed the presence of high concentration of AFB_1 in both dried meat and dried fish sold in the sampled markets. However, consumption of these food items as protein sources pose a great health hazard to consumers.

It is therefore recommended that regulation standards should be put in place and enforced to promote hygienic processing, transportation and storage of these animal products. There is also a need to educate both the traders and the consumers on the risk involved in consumption of such contaminated products

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