INCIDENT OF MYCOTOXINS IN Mouldy Smoked Dried Fish and Meat (Kundi) Marketed in Southwestern Nigeria

Fatima O. Wambai1, Olusola Awotula1, Oluwapelumi A. Oyedele2, Chibundu N. Ezekiel2, Adeyinka O. Akintunde1, Grace O. Tayo*

Address(es):
1Department of Agriculture, Babcock University, Ilishan Remo, Ogun State, Nigeria.
2Department of Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria.

*Corresponding author: tayog@babcock.edu.ng

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 B1. Aflatoxin B1, G1 and G2 were below detectable limits. About 13% of the samples had aflatoxin B1 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 Adedanjo, 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 tinsa (Obanu et al., 1987). Dried meat also known as kundi, tinsa and bandu in Yoruba and Hausa respectively is a cheap, poor quality meat derived from various types of livestock (donkeys, horses, camel and buffalo) (Adeneye, 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 (Ekhotar et al., 2018). Contamination can originate from soil dust or/and from combustion in industry and from forest fires as well as forest fires and volcanic eruptions (Chaturvedi 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 niacin and riboflavine, 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 to mould growth and mycotoxin production. Mycotoxins are secondary metabolites produced by fungi in various food commodities and feed (Adeneye, 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, Aspergillus cerealis (Ezekiel et al., 2014). Aflatoxins are grouped into namely AFB1, AFB2, AFG1, AFG2, AFB1 is considered to be the most potent metabolite and has been classified under class 1 human carcinogen by International Agency for Research on Cancer (IARC), it is highly hepatotoxic, 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 -20°C 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% chloranithicol and streptomycin. All isolations were performed in triplicates and the inoculated plates were incubated for 3–5 days at 31°C. All colonies were counted and fungal load expressed as Log10 colony forming units per gram (log10 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 31°C, the macro
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 fluorescence resulting from 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

| MONTH 1 | FREQUENCY | PERCENTAGE (%) |
|---------|-----------|----------------|
| Aspergillus section Flavi | 48 | 48.98 |
| Aspergillus niger – clade | 30 | 30.61 |
| Rhizopus spp. | 20 | 20.41 |

| MONTH 2 | FREQUENCY | PERCENTAGE (%) |
|---------|-----------|----------------|
| Aspergillus section Flavi | 37 | 56.92 |
| Aspergillus niger – clade | 25 | 38.46 |
| Rhizopus spp. | 3 | 4.62 |

| MONTH 3 | FREQUENCY | PERCENTAGE (%) |
|---------|-----------|----------------|
| 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

| Weeks | Ns | Na | Concentration (ng/g) | X ± SD (ng/g) |
|-------|----|----|----------------------|--------------|
| 1     | 10 | 0 | 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, Na – number of samples analyzed, X – number of samples contaminated, X – mean, SD – standard deviation

Table 3: Occurrence of Aflatoxin B$_1$ in Dried Meat

| MONTH 1 | Ns | Na | Concentration (ng/g) | X ± SD (ng/g) |
|---------|----|----|----------------------|--------------|
| 15      | 5  | 5  | 3.10–6.93            | 4.66±1.52    |
| 15      | 5  | 5  | 1.92–5.09            | 3.49±1.24    |
| 15      | 5  | 5  | 2.60–18.94           | 8.53±6.82    |

ALL SAMPLES 45 15 1.92–18.94 5.56±4.40

Legend: Ns – number of samples obtained, Na – number of samples analyzed, X – number of samples contaminated, 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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