

## Survey of aflatoxins and fungi in some commercial breakfast cereals and pastas retailed in Ogun State, Nigeria

Ezekiel, C.N.<sup>1,\*</sup> and Sombie, J.I.<sup>1</sup>

<sup>1</sup>Mycology/Mycotoxicology Research Unit, Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Ogun State, Nigeria.

\* [chauguez@gmail.com](mailto:chauguez@gmail.com)

**Abstract:** The incidence of aflatoxin and fungal load were determined in 30 samples of five commonly consumed maize-based breakfast cereals (cornflakes and golden morn) and wheat-based pastas (macaroni, noodles and spaghetti) retailed within Ogun State, Nigeria. Aflatoxin was quantified in all food samples at concentrations ranging 0.8–3.5 ppb (mean = 1.3 ppb). Golden morn had a mean aflatoxin concentration of 2.3 ppb, a level significantly ( $p < 0.05$ ) higher than all other food commodities. Furthermore, the maize-based breakfast cereals (mean = 1.7 ppb) had significantly ( $p < 0.05$ ) higher aflatoxin concentrations than the wheat-based pastas (mean = 1.2 ppb). Obviously, all the food commodities were contaminated with aflatoxin far below the stipulated 20 ppb limit. In contrast to the aflatoxin data obtained, none of the moulds belonging to the aflatoxigenic species was recovered from any of the commodities. However, fungi belonging to the *Aspergillus*, *Fusarium* and *Penicillium* genera were broadly identified alongside some unidentifiable moulds in 46.7% of the food samples. Aflatoxin contamination in processed foods such as those investigated in this study may not necessarily pose an acute health risk to consumers; however, a long term effect is likely.

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### 1. Introduction

Aflatoxins [e.g. aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>] are toxic secondary metabolites produced by some *Aspergillus* species when they invade and colonize agricultural commodities including cereals (e.g. maize and wheat). These naturally occurring toxic compounds are known to be carcinogenic, hepatotoxic, immunotoxic and teratogenic in humans and many animal species (IARC, 2002). Contamination of food by aflatoxin producing species (e.g. *Aspergillus flavus*) and consequent release of aflatoxins in the food often lead to rejection of the food if toxin levels are above stipulated limits, and severe health risks when such foods are consumed (Williams *et al.*, 2004). Consequently, high exposure to aflatoxins have been reported in populations residing in aflatoxin-endemic countries alongside increased incidence of acute hepatic necrosis, resulting later in cirrhosis, or hepatocellular carcinoma (Turner *et al.*, 2002; Egal *et al.*, 2005; Ezekiel *et al.*, 2014; Abia *et al.*, 2013b).

Breakfast cereals commonly consumed in Nigeria are cornflakes, golden morn and oats while the pastas include macaroni, noodles and spaghetti. These commodities serve as quick foods for children and adults in more than one third of the homes in Nigeria and beyond (Murphy *et al.*, 2006). In Nigeria, a number of indigenous industries engage in the production of all the above-mentioned breakfast

cereals and pastas excluding oats. The breakfast cereals produced in Nigeria are made from maize while the pastas are wheat-based. These food commodities are mainly produced by processing maize and wheat, and processing involves sorting and milling of dry grains, and addition of some adjuncts: honey, sugar, chocolate and dried raisins.

Regardless of the wide consumption of these two groups of food by Nigerians, little or no data are available as regards mycotoxin levels in the commodities; the need for this study. However, aflatoxin and fungi contamination of maize and maize-based products in Nigeria has been well studied and documented (Adebajo *et al.*, 1994; Atehnkeng *et al.*, 2008; Jimoh and Kolapo, 2008; Ezekiel *et al.*, 2012; Perrone *et al.*, 2014) but scanty data exist for wheat from Nigeria. The major fungi previously reported to invade and contaminate maize and its products include *Aspergillus*, *Fusarium* and *Penicillium* (Adebajo *et al.*, 1994; Atehnkeng *et al.*, 2008; Jimoh and Kolapo, 2008; Ngoko *et al.*, 2008; Ezekiel *et al.*, 2012) while those reported for wheat include *Aspergillus* section *Nigri* and *Flavi* species (Rashid *et al.*, 2008; Riba *et al.*, 2010; Dovicicova *et al.*, 2012). In this study, we screened a small number of breakfast cereals and pastas retailed in Ogun State for aflatoxin levels and fungal load with the aim of providing preliminary useful data on the aflatoxin status of these foods consumed in many homes.

## 2. Materials and methods

### 2.1 Sampling and samples

Surveys were conducted in two big markets in Ogun State to determine the various kinds of breakfast cereals and pastas commonly retailed to consumers. The identified products were categorized into five and they include: cornflakes (n = 6), golden morn (n = 3), macaroni (n = 3), noodles (n = 9) and spaghetti (n = 9). Cornflakes and golden morn are made from maize and regarded as breakfast cereals while macaroni, noodles and spaghetti are wheat-based and considered as pastas. The manufacturer details of each product including brand name, batch numbers, production and expiry dates, and primary ingredient were obtained from the packaging of each product but are not disclosed here due to the confidential nature of these details.

A total of 30 samples (each weighing approximately 500 g) were randomly collected in January 2014, each sample representing a pack of a product. Each sample was quartered and about 50 g was taken and ground in a commercial Blender (Waring Commercial Blender 8010BU, Model HGBTWT, Connecticut, USA). Each ground sample was subdivided into two parts: Part A, for aflatoxin analysis by ELISA, and Part B for mycological analysis.

### 2.2 Determination of aflatoxins in food

All part A subsamples were analyzed for the presence of aflatoxins (total aflatoxins = sum of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>) by the Competitive Direct (CD) ELISA method. The AgraQuant<sup>®</sup> Total Aflatoxin Assay 4/40 kit (Product no. COKAQ1000; Romer Labs, Singapore) was used. All reagents including LC grade methanol were purchased from Sigma Aldrich (St Louis, MO, USA).

Extraction and testing of aflatoxins were performed on each sample according to manufacturer instructions in the ELISA kits. Briefly, 5 g of each representative sample was weighed into a 250 ml conical flask and extracted with 25 ml of 70% methanol (1:5 w/v) on an orbital shaker for 10 min at ambient temperature. The mixture was filtered through a 110 mm No.1 Whatman filter paper into a 40 ml tube.

An aliquot (50 µl) of each filtrate representing a sample was then dispensed into dilution wells and 100 µl of AgraQuant<sup>®</sup> aflatoxin conjugate was added to each sample/filtrate in the dilution wells and mixed. About 100 µl of this mixture was then dispensed into antibody-coated microwells and incubated at ambient temperature for 15 min. Subsequently, the contents of the incubated microwells were emptied and washed thrice with distilled water to rinse off all remaining mixture. A

100 µl aliquot of the substrate was further added to the washed wells and the wells were observed for colour development after incubation at room temperature for 5 min. To end the reaction, 100 µl of the stop solution was added to each microwell. All microwells were then placed in an ELISA microplate reader (BioTek-ELX800) with a 450 nm filter to determine the concentration of total aflatoxins in each sample. The total aflatoxin estimates in each sample was obtained by extrapolation from the standard curve.

### 2.3 Mycological analysis of food samples

Each of the part B subsample was subjected to mycological analysis and moulds were isolated by the dilution plating technique described by Samson *et al.* (1995). Briefly, 10 g of each subsample was suspended in 90 ml of sterile distilled water, homogenized for 1 min and surface-plated in triplicate on full strength potato dextrose agar [PDA, 9.75 g/l PDB (Difco) and 20 g/l bacto agar] supplemented with 0.001% chloramphenicol and streptomycin sulphate. The inoculated plates were incubated at 25 °C for 5–7 days. All fungal colonies were counted and species of *Aspergillus*, *Fusarium*, *Penicillium* and *Talaromyces* were recorded as belonging to their specific genera while all other moulds including the Mucorales were reported as “Other”. Mould load per sample was derived from plate counts and expressed as a logarithm of colony-forming units per gram of sample (Log<sub>10</sub>Cfu/g).

All isolates were purified by the single colony transfer technique on freshly prepared PDA and malt extract agar. The pure isolates were morphologically identified by assessing macro- and microscopic characters in line with appropriate keys (Frisvad and Samson, 2004; Leslie and Summerell, 2006; Pitt and Hocking, 2009; Samson *et al.*, 2010, 2011).

### 2.4 Data analysis

All data obtained from this study was analyzed using SPSS<sup>®</sup> (Windows version 15.0, SPSS, IL, USA). Means for total aflatoxin concentrations and fungal load were calculated for each product. All means were tested for significance at 95% confidence level using one way ANOVA and separated by Duncan's Multiple Range Test.

## 3. Results

### 3.1 Incidence of aflatoxin in food

All 30 food commodities analyzed in this study were contaminated with aflatoxins at concentrations ranging 0.8–3.5 ppb (mean = 1.3 ppb) (Table 1). Cornflakes (mean = 1.4 ppb) and golden morn (mean = 2.3 ppb), two breakfast cereals made from maize, had higher aflatoxin levels than macaroni (mean = 1.1 ppb), noodles (mean = 2.2 ppb) and spaghetti (mean = 1.2 ppb) which are grouped as pastas and

derived from wheat. However, only the mean aflatoxin concentration observed in golden morn was significantly ( $p < 0.05$ ) different from all other food commodities. Grouping the commodities based on their basal ingredient/grain (Table 2) showed that the maize-based foods (breakfast cereals; range = 0.8–3.5 ppb; mean = 1.7 ppb) had significantly ( $p < 0.05$ ) higher aflatoxin concentrations than the wheat-based pastas (range = 0.8–1.6 ppb; mean = 1.2 ppb). Overall, aflatoxin levels in all the food commodities were below the 20 ppb regulation for aflatoxins in food.

Table 1. Distribution of aflatoxin in five types of breakfast cereals and pastas retailed in Ogun State, Nigeria.

Commodities <sup>1</sup>	N <sup>2</sup>	Aflatoxin <sup>3</sup> concentration (ppb)	
		Range	Mean <sup>4</sup> ± SE
Golden Morn	3/3	1.5 – 3.5	2.3 <sup>a</sup> ± 0.6
Cornflakes	6/6	0.8 – 2.8	1.4 <sup>b</sup> ± 0.3
Macaroni	3/3	1.0 – 1.3	1.1 <sup>b</sup> ± 0.1
Noodles	9/9	0.9 – 1.3	1.2 <sup>b</sup> ± 0.1
Spaghetti	9/9	0.8 – 1.6	1.2 <sup>b</sup> ± 0.1
Total	30/30	0.8 – 3.5	1.3 ± 0.1

<sup>1</sup>Commodities are grouped as breakfast cereals (cornflakes and golden morn) and pastas (macaroni, noodles and spaghetti).

<sup>2</sup>Number of samples contaminated with aflatoxins.

<sup>3</sup>Aflatoxin refers to sum of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>.

<sup>4</sup>Mean values with different superscript alphabets are significantly different at  $\alpha = 0.05$ .

Table 2. Incidence of aflatoxin in breakfast cereals and pastas grouped by their cereal-base.

Grain <sup>1</sup> type	N <sup>2</sup>	Aflatoxin <sup>3</sup> concentration (ppb)	
		Range	Mean <sup>4</sup> ± SE
Maize	9	0.8 – 3.5	1.7 <sup>a</sup> ± 0.3
Wheat	21	0.8 – 1.6	1.2 <sup>b</sup> ± 0.1

<sup>1</sup>Grains that constitute main ingredient for breakfast cereal & pasta production.

<sup>2</sup>Number of samples analyzed.

<sup>3</sup>Aflatoxin refers to sum of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>.

<sup>4</sup>Mean values with different superscript alphabets in a column are significantly different at  $\alpha = 0.05$ .

### 3.2. Incidence of moulds in food commodities

Only 14 (46.7%) out of the 30 samples were contaminated by fungal propagules. The incidence and distribution of moulds in the contaminated samples are given in Table 3. Fungal load was higher in breakfast cereals (mean = 300 CfU/g) than in pastas

(163.6 CfU/g) though at an insignificant ( $p = 0.21$ ) level. Also, no significant difference was observed in mould load across food commodities. Three major fungal genera were identified in the samples: *Aspergillus*, *Fusarium* and *Penicillium*. *Aspergillus* species (incidence = 56.3%) was the most prevalent fungi recovered from the pastas while species of *Penicillium* (incidence = 44.5%) occurred the most in breakfast cereals. Generally, *Fusarium* had the least incidence (6.3–22.2%). The *Aspergillus* isolates obtained from the samples were identified as either *Aspergillus niger*-clade (black aspergilli) according to the descriptions in Pitt and Hocking (2009) or regarded as *Aspergillus* spp. in cases where their taxonomy was confusing due to the adoption of the morphological method. The *Aspergillus niger*-clade (32%) was dominant in all food commodities than all other *Aspergillus* species (8%). None of the section *Flavi* species was found. All unidentifiable fungi recovered from the samples were regarded as “Other” (incidence = 28%).

## 4. Discussion

Contamination of food by aflatoxigenic species and consequent release of mycotoxins (e.g. aflatoxins) in the food material continues to arouse research interests worldwide due to the potential health risks associated with the ingestion of these natural toxins (Atanda *et al.*, 2013). In Nigeria, just like other sub-Saharan African countries, the case is even more critical because climatic conditions, poor agricultural practices and poor systems for post-harvest storage of grains tend to favour the dispersal and proliferation of the aflatoxigenic species. This often leads to an increased mycotoxin level in finished/processed agricultural commodities because food processors tend to acquire contaminated grains as raw materials for use in the industry, though food processing techniques may help to reduce toxin levels. It is further speculated that control and monitoring of food products for mycotoxin compliance is inadequate in developing countries (e.g. sub-Saharan Africa), especially in foods produced locally such as those made by indigenous industries (Williams *et al.*, 2004).

In this study, we found that all tested food samples contained aflatoxins but at levels far below the set limits. The individual and mean aflatoxin levels in each of the commodities were similar to the AFB<sub>1</sub> level (1.18 µg/kg) in Algerian semolina (Riba *et al.*, 2010) but higher than aflatoxin levels (<1 ng/g) in corn- and wheat-based breakfast cereals from Canada (Tam *et al.*, 2006). On the other hand, mean aflatoxin levels reported here were lower than the mean levels reported in: a cornflakes sample (6.1 µg/kg) from Egypt (Nogaim *et al.*, 2011), macaroni

(5.91 µg/kg), noodles (7.35 µg/kg) and spaghetti (9.12 µg/kg) from Pakistan (Iqbal *et al.*, 2013). This is a plus to the industries and consumers considering the fact that the food commodities passed trade regulations; hence, a single dose may not be an instant source of acute aflatoxicosis. However, when the daily/continuous consumption of these products is considered, especially in the case of children who are

the major consumers of the cereals, health risks are likely. This is due to the known facts about aflatoxin exposure: (1) chronic, subclinical exposure occurs over time though it does not lead to symptoms as dramatic as acute aflatoxicosis; (2) children are particularly affected leading to their stunted growths and delayed development (Gong *et al.*, 2002, 2003; Turner *et al.*, 2003).

Table 3. Incidence of fungi in breakfast cereals and pastas retailed in Ogun State, Nigeria.

Commodities	N <sup>1</sup> (%)	Mean <sup>2</sup> fungal load (Cfu/g)	Percentage occurrence of fungi			
			<i>Aspergillus</i>	<i>Fusarium</i>	<i>Penicillium</i>	Other
Cornflakes	1/6 (16.7)	600	0.0	16.7	66.7	16.7
Golden morn	2/3 (66.7)	150	33.3	33.3	0.0	33.3
Mean <sup>2</sup> for breakfast cereals <sup>3</sup>	3/9 (33.3)	300 <sup>a</sup>	11.1 <sup>b</sup>	22.2 <sup>a</sup>	44.5 <sup>a</sup>	22.2 <sup>a</sup>
Macaroni	3/3 (100.0)	233	71.4	0.0	0.0	25.0
Noodles	4/9 (44.5)	125	20.0	0.0	20.0	28.6
Spaghetti	4/9 (44.5)	150	75.0	25.0	0.0	0.0
Mean <sup>2</sup> for pastas <sup>3</sup>	11/21 (52.4)	163.6 <sup>a</sup>	56.3 <sup>a</sup>	6.3 <sup>b</sup>	6.3 <sup>a</sup>	31.3 <sup>a</sup>
Total	14/30 (46.7)	-	40.0	12.0	25.0	28.0

<sup>1</sup>Number and percentage of contaminated samples.

<sup>2</sup>Mean values with different superscript alphabets in a column are significantly different at  $\alpha = 0.05$ .

<sup>3</sup>Categories of commodities.

The significantly higher aflatoxin levels in maize-based cereals compared to levels in the wheat-based pastas is in line with previous studies that have reported maize and maize-products to be more prone to aflatoxin contamination than other crops excluding groundnut (Bandyopadhyay *et al.*, 2007; Ezekiel *et al.*, 2012; Warth *et al.*, 2012; Abia *et al.*, 2013a). The low aflatoxin levels recorded in the commodities may either be as a result of one or more of these factors: industries sourced for quality raw materials (grains) due to strict monitoring by regulatory agencies, processing techniques (e.g. sorting and gritting) helped to reduce toxin levels considerably, or effective internal control of finished products by industries' quality assurance units. It is obvious that processing and proper packaging of products must have contributed the most to the non-recovery of *Aspergillus* section *Flavi* species from the commodities after repeated isolations. For the recovered moulds, the fungal counts were very low.

This study re-emphasizes the need for food processors to painstakingly source for quality grains as raw materials for breakfast cereal and pasta production because regardless of commodity produced, if the starting ingredient (grains) are contaminated with aflatoxins, the finished product will also contain aflatoxins. Furthermore, it should be noted that high level of aflatoxins in food commodities may lead to a higher level of exposure and increased incidence of acute hepatic necrosis, resulting later in cirrhosis, or hepatocellular

carcinoma in consumers (Egal *et al.*, 2005; Turner *et al.*, 2005). Since humans are not immune to the acute toxic effects of aflatoxins; though the adult population has a high tolerance for aflatoxin exposure and rarely succumb to acute aflatoxicosis (Williams *et al.*, 2004); more effort should be channeled into the enforcement of regulatory limits for foods produced by indigenous companies in order to maintain a high standard.

#### Corresponding Author:

Dr. Chibundu N. Ezekiel  
Department of Biosciences and Biotechnology,  
Babcock University, Ilishan Remo,  
Ogun State, Nigeria.  
[chauguez@gmail.com](mailto:chauguez@gmail.com)

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