

Occurrence of Aflatoxin in Some Food Commodities Commonly Consumed in Nigeria

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Abstract

Aflatoxicosis is a public health problem in Nigeria like other tropical and sub-tropical regions of the world. Control of aflatoxin contamination requires thorough risk assessment, monitoring, quality control and empirical data. This study assayed total aflatoxin levels, identified and quantified four aflatoxin types in five food commodities commonly consumed in the six geopolitical zones of Nigeria. The food materials: *Zea mays*, *Colocynthis citrullus*, *Capsicum frutescens*, *Irvingia gabonensis* and *Arachis hypogaea* were obtained from Watt market in Calabar urban. ELISA method was used for total aflatoxin, HPLC for aflatoxin types, AOAC for moisture. All (100%) the samples were contaminated with aflatoxin. Contamination was highest in *Irvingia gabonensis* ($63.40 \pm 1.79 \mu\text{g/kg}$) and least in *Zea mays* ($3.20 \pm 0.12 \mu\text{g/kg}$) ($p < 0.05$). Except for *Irvingia gabonensis* and *Colocynthis citrullus*, total aflatoxin was within safe intake level of the Nigerian regulatory authority (National Agency for Food and Drug Administration and Control {NAFDAC}). All four aflatoxin types occurred in *Irvingia gabonensis*, *Capsicum frutescens* and *Colocynthis citrullus*; none was detected in *Arachis hypogaea*. AFB₁ contamination was highest in *Irvingia gabonensis* ($11.71 \pm 0.10 \mu\text{g/kg}$) followed by *Capsicum frutescens* ($1.21 \pm 0.01 \mu\text{g/kg}$); AFB₂ ranged from 0.00 ± 0.00 - $2.43 \pm 0.05 \mu\text{g/kg}$, AFG₁ 0.00 ± 0.00 - $3.73 \pm 0.04 \mu\text{g/kg}$, and AFG₂ 0.00 ± 0.00 - $0.54 \pm 0.01 \mu\text{g/kg}$ ($p < 0.05$). Only *Irvingia gabonensis* exceeded the limit of AFB₁ specified by NAFDAC for human foods. Moisture content varied widely ($3.23 \pm 0.03\%$ - $10.37 \pm 0.19\%$). The trend in the occurrence of aflatoxins in the food samples was directly proportional ($r = 0.91$) to their moisture contents. Food commodities sold in Calabar carry potential health hazard. Improved handling through food processing, preservation and storage can minimize aflatoxins in foodstuffs and ensure sustainable quality of food supply.

Keywords: aflatoxicosis, carcinogenicity, food safety, mycotoxigenic fungi, NAFDAC

1. Introduction

The safety of food and feed has been a major concern of nations especially in recent years as more knowledge is gathered on the occurrence of natural toxins in food stuffs, fertilizers, animal feed and edible plant materials. Naturally occurring toxins have been characterized by the World Health Organization (WHO, 2002) as significant sources of food borne illnesses. Of the natural food toxins, the Food and Agriculture Organization (FAO) has estimated that mycotoxins (fungal toxins) alone contaminate about 25% of agricultural products worldwide resulting in huge losses for farmers (Smith, Solomons, Lewis, & Anderson, 1994; Wu, 2007). The biochemical properties of mycotoxins are diverse, and their toxic effects are exceedingly variable. Mycotoxins are carcinogenic, tremorogenic, haemorrhagic, genotoxic, teratogenic, nephrotoxic, hepatotoxic and immunotoxic (Refai, 1988; Hosseini & Bagheri, 2012).

Out of the about 300 mycotoxins so far known, aflatoxin is the most studied, because of its common occurrence, high potency and toxicity to man and animals (SP-IPM, 2009). Aflatoxins are associated with high incidences of liver cancer in Africa and elsewhere and are thought to exacerbate diseases such as hepatitis B virus-induced liver cancer and HIV/AIDS (Shepherd, 2008). Other health effects of aflatoxins in animals and humans include

reduced growth rate, weakened immune system and death (Eaton & Groopman, 1994). At least thirteen (13) different types of aflatoxins are produced in nature with aflatoxin B₁ considered as the most toxic and therefore of particular public health importance.

In recognition of the many dangers of aflatoxins, researchers and farmers around the world are seeking an understanding of how to manage the contamination dangers resulting from them. Meanwhile, policy makers are in the process of balancing food safety with food availability, a task requiring a thorough risk assessment and monitoring as well as empirical data. In view of the fact that foods from maize (*Zea mays*), melon seed (*Colocynthis citrullus*), ground nut (*Arachis hypogea*), bush mango (*Irvingia gabonensis*) and red pepper (*Capsicum frutescens*) are consumed in a high rate in Nigeria, it is important to ensure that foods consumed are of premium quality of zero or minimal levels of aflatoxin and other contaminants. This is because people are as healthy as the food they eat. In addition to this, food adds to the economical income of man but unacceptable aflatoxin levels could pose a threat to this opportunity. This work was designed to assay for the aflatoxin contamination associated with some foodstuffs sold in Calabar urban with a view to enlightening the consumers on the need for proper food handling.



Figure 1. *Irvingia gabonensis*



Figure 2. *Capsicum frutescens*



Figure 3. *Colocynthis citrullus*



Figure 4. *Arachis hypogea*



Figure 5. *Zea mays*

2. Materials and Methods

2.1 Collection of Food Materials and Preliminary Preparation of Samples

One (1) kg each of the five different food items: *Zea mays* (maize), *Colocynthis citrullus* (melon seed), *Capsicum frutescens* (red pepper), *Irvingia gabonensis* (bush mango) and *Arachis hypogea* (groundnut) were purchased in their dried forms from five different shops in Watt Market in Calabar urban in January, 2013. The food materials were transported to the Research Laboratory in the Department of Biochemistry, University of Calabar where all extraneous materials were removed. Each food material type was ground into powder and thoroughly mixed using a milling machine (MF-10 Basic IKA Werke, USA). Two (2) kg of each powdered

sample was pooled and packaged in a sterile airtight self-adhesive polyethene pouch, labeled properly and stored in a glass chamber maintained under UV air sterilizer pending aflatoxin analysis and identification within 12 hours.

2.2 Determination of Moisture Content

The moisture content of each sample was determined using Automated Moisture Analyzer (Sartorius MA 150, Germany) as described by the Association of Official Analytical Chemists (AOAC, 2006). The method is based on loss of moisture upon drying at 105 °C.

2.3 Preparation of Samples for Total Aflatoxin Analysis

For the extraction of non-coloured samples, five hundred (500) g each of the samples of *Zea mays* and *Colocynthis citrullus* was weighed into a clean neogen cup, 1L of distilled water was added and covered tightly. The samples were allowed to settle after which the top layer of the extract was filtered through a Whatman No. 1 filter paper (150mm). The filtrate collected was then concentrated in a rotary evaporator (RE 300B Serial No. R000010551, UK) *in vacuo* at 40 ± 2 °C and used for total aflatoxin analysis.

For the extraction of naturally coloured samples, five hundred (500) g each of the composite samples of *Capsicum frutescens*, *Irvingia gabonensis* and *Arachis hypogea* was weighed into a clean neogen cup, 1.25 L of methanol/tween water (70:30) was added, covered tightly and placed on a laboratory shaker set at 250 rpm for 3minutes 30seconds. The samples were allowed to settle and the top layer of the extract filtered through a Whatman No. 1 filter paper (150mm). The filtrate collected was then concentrated in the rotary evaporator *in vacuo* at 40 ± 2 °C and used for total aflatoxin analysis.

2.4 Determination of Total Aflatoxin

Total aflatoxin content was determined using the AgraQuant assay kit (Romer Labs® Order No.COKAQ1100, Singapore). The AgraQuant assay kit procedure is based on a direct competitive enzyme-linked immunosorbent assay (ELISA) method as described by Ayar et al. (2007). The total aflatoxin concentration was read at 450 – 630 nm.

2.5 Preparation of Samples for Identification and Quantification of Individual Aflatoxins

Two hundred (200) g each of the samples was weighed into a 1L conical flask and 500 ml of 80% methanol was added and blended for 3 minutes. The homogenized sample was transferred into a 1 L conical flask and let to settle for 10 minutes. The supernatant layer was transferred into a 100ml centrifuge tube and centrifuged at 3000 rpm for 10minutes using a refrigerated centrifuge (Eppendorf AG 22331 Hamburg, Germany). The supernatant of the spun sample was transferred into a 500 ml separating funnel, 50 ml of 10% NaCl solution was added, followed by 50 ml hexane. The mixture was shaken for 1 minute and the organic layer discarded. To the aqueous layer collected, 50 ml of dichloromethane was added and shaken for 2 minutes. The dichloromethane layer was then passed through anhydrous sodium sulphate to remove the traces of moisture. The dichloromethane extraction was repeated twice and the fractions were combined and evaporated in the rotary evaporator under vacuum at 40 ± 2 °C. This was transferred into a 5ml volumetric flask and made up to the mark with dichloromethane. Two (2) ml was drawn into a vial and evaporated in the rotary evaporator at 40 ± 2 °C. The residue was then taken for HPLC analysis.

2.6 Identification and Quantification of Individual Aflatoxins

The residue obtained from the concentrated extract was each dissolved in 1ml mobile phase and injected into the High Performance Liquid Chromatography (HPLC) column. A sensitive UV detector set at 365 nm was used to detect the aflatoxin types, which were eluted as sharp peaks within 4-7 minutes. The concentration of each aflatoxin type (AFB₁, AFB₂, AFG₁ and AFG₂) was determined using the peak area ratios as described by AOAC (2006) and Herzallah (2009). The absorbance of each aflatoxin species was read at 365 nm.

2.7 Statistical Analysis

The data obtained were subjected to analyses using the one-way analysis of variance (ANOVA) in SPSS statistical package; LSD was used for multiple comparisons. Statistical significance was accepted at 5% probability level or less.

3. Results

The *Irvingia gabonensis* sample had the highest ($10.37 \pm 0.19\%$), while *Zea mays* had the least ($3.23 \pm 0.03\%$) moisture content ($p < 0.05$). Low moisture content was also recorded in *Colocynthis citrullus* and *Arachis hypogea* ($4.05 \pm 0.03\%$ and $4.48 \pm 0.01\%$ respectively); *Capsicum frutescens* had a moderate ($6.75 \pm 0.08\%$)

level of moisture. The results also showed significantly different contents of total aflatoxin among the food commodities. Again, *Irvingia gabonensis* had the highest ($63.40 \pm 1.79 \mu\text{g/kg}$), while *Zea mays* had the least ($3.20 \pm 0.12 \mu\text{g/kg}$) total aflatoxin content. The total aflatoxin content in the other samples were: $8.00 \pm 0.06 \mu\text{g/kg}$ in *Capsicum frutescens*, $6.10 \pm 0.06 \mu\text{g/kg}$ in *Colocynthis citrullus* and $3.40 \pm 0.01 \mu\text{g/kg}$ in *Arachis hypogea* (Table1).

The aflatoxin concentrations in the five different foodstuffs evaluated correlated directly ($r = 0.91$) with the moisture contents in the foodstuffs (Figure 6).

Aflatoxin B₁ (AFB₁) concentration was significantly higher ($p < 0.05$) in *Irvingia gabonensis* ($11.71 \pm 0.10 \mu\text{g/kg}$) than the other four samples. AFB₁ was $1.21 \pm 0.01 \mu\text{g/kg}$ in *Capsicum frutescens*, $0.82 \pm 0.02 \mu\text{g/kg}$ in *Colocynthis citrullus* but not detected in the *Zea mays* and *Arachis hypogea* samples. Similarly, *Irvingia gabonensis* had the highest ($p < 0.05$) AFB₂ content (2.43 ± 0.05). Other results for AFB₂ were: $0.34 \pm 0.01 \mu\text{g/kg}$ in *Capsicum frutescens*, $0.05 \pm 0.01 \mu\text{g/kg}$ in *Colocynthis citrullus*, but not detectable in *Zea mays* and *Arachis hypogea*. The AFG₁ was significantly ($p < 0.05$) higher in *Capsicum frutescens* ($3.73 \pm 0.04 \mu\text{g/kg}$) than in the other samples ($1.85 \pm 0.03 \mu\text{g/kg}$ in *Irvingia gabonensis*, $0.39 \pm 0.01 \mu\text{g/kg}$ in *Colocynthis citrullus*, and $0.19 \pm 0.01 \mu\text{g/kg}$ in *Zea mays*); *Arachis hypogea* did not show any detectable level of AFG₁. The AFG₂ was highest in *Zea mays* ($0.54 \pm 0.01 \mu\text{g/kg}$) ($p < 0.05$) compared to the other four samples ($0.20 \pm 0.20 \mu\text{g/kg}$ in *Capsicum frutescens*, $0.14 \pm 0.01 \mu\text{g/kg}$ in *Irvingia gabonensis* and $0.09 \pm 0.00 \mu\text{g/kg}$ in *Colocynthis citrullus*). Again, AFG₂ was not detected in *Arachis hypogea* (Figure 7).

Table 1. Moisture and total aflatoxin contents of the food samples

Food samples	Moisture (%)	Total AFs ($\mu\text{g/kg}$)
<i>Zea mays</i>	3.23 ± 0.03	3.20 ± 0.12
<i>Colocynthis citrullus</i>	$4.05 \pm 0.03^*$	$6.10 \pm 0.06^*$
<i>Capsicum frutescens</i>	$6.75 \pm 0.08^{*,a}$	$8.00 \pm 0.06^*$
<i>Irvingia gabonensis</i>	$10.37 \pm 0.19^{*,a,b}$	$63.40 \pm 1.79^{*,a,b}$
<i>Arachis hypogea</i>	$4.48 \pm 0.01^{*,a,b}$	$3.40 \pm 0.01^{a,b,c}$

* = significantly different from *Z. mays* at $p < 0.05$; a = significantly different from *C. citrullus* seed at $p < 0.05$
b = significantly different from *C. frutescens* at $p < 0.05$; c = significantly different from *I. gabonensis* at $p < 0.05$
Values are expressed as mean \pm SEM, n=3 (where SEM = standard error of mean).

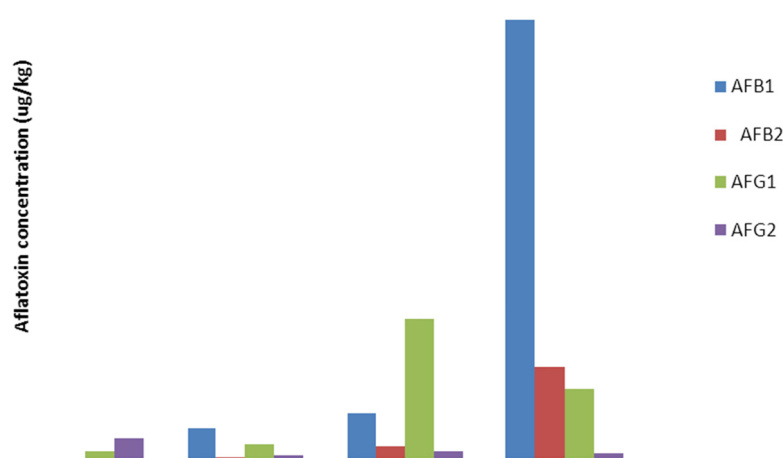


Figure 7. Aflatoxins B₁, B₂, G₁ & G₂ Concentration in the food samples

* = $p < 0.05$ vs B₁; a = $p < 0.05$ vs B₂; b = $p < 0.05$ vs G₁; Values are expressed as mean \pm SEM, n = 3 (where SEM = standard error of mean).

4. Discussion

This study assayed for the total aflatoxin content and concentration of four aflatoxin types (AFB₁, AFB₂, AFG₁, and AFG₂) in five locally available food commodities commonly used in food preparation in Nigeria. The food materials were: common maize (*Zea mays*), melon seed (*Colocynthis citrullus*), red pepper (*Capsicum frutescens*), bush mango (*Irvingia gabonensis*) and groundnut (*Arachis hypogea*). The results showed that all (100%) the food samples were contaminated with aflatoxin. It was further observed that except for bush mango and melon seeds, the levels of total aflatoxin in the food commodities were generally below the maximum allowable limits (10 µg/kg for pepper, and 4 µg/kg for others) specified by the European Commission (AESAN, 2011), which is also currently being used by the National Agency for Food and Drug Administration and Control (NAFDAC), in Nigeria. This observation agrees with the findings from other studies (Adebayo-Tayo, Onilude, Ogunjobi, Gbolagade, & Oladapo, 2006; Romagnoli, Meena, Gruppioni, & Bergamini, 2007; Russell & Peterson, 2007). However, levels of total aflatoxins below the levels found in the present study have also been reported in some foodstuffs (Zinedine et al., 2006).

The high levels of total aflatoxin found in some of the food materials used in this study may be attributed to some biotic and abiotic factors such as temperature, relative humidity, moisture content, food chemical composition, storage duration and insect attack. Studies by Simsek et al. (2002) showed that temperature favourable for aflatoxin production is 25-30 °C and relative humidity 97-99%. Also, Ross et al. (1979) had earlier reported that if both temperature (20-38°C) and moisture (16-24%) are favourable for *Aspergillus flavus*, aflatoxin can be produced within 48 hours.

The level of moisture found in some of the food materials used in this study may be responsible for the growth of microorganisms with subsequent production of aflatoxins. Smith and Moss (1995) had shown that moisture determines whether microbes can colonize a substrate or not. The high moisture content in some of the food materials used in this study may be as a result of high humidity (>70%) and high temperature (>25%), which are characteristic of the tropical and sub-tropical regions of the world where Nigeria is located.

Also in this study, bush mango showed the highest level of total aflatoxin, followed by red pepper, melon seed and groundnut, while maize had the least. The trend in the occurrence of aflatoxins in these food samples is directly proportional to their moisture contents. It is therefore most probable that the differences in the moisture content of these food materials were responsible for the levels of total aflatoxin found in them. Maize which had the least moisture content also had the lowest total aflatoxin content. According to Sakai et al. (1984) and Schetzki and Ong (2001), substrate chemical composition is an important factor in aflatoxin contamination. Maize is a commodity with low lipid content while bush mango, melon seed and groundnut are oil seeds and contain high levels of lipid. It is relatively easier to dehydrate a food commodity with low lipid content than that with high lipid content. Hence, a higher level of moisture in the bush mango, melon seed and groundnut, leading to higher levels of total aflatoxins in them than in maize. In this study, a statistical correlation was found between aflatoxin concentration and moisture content of the food samples.

The results of this study also showed that AFB₁, AFB₂, AFG₁ and AFG₂ were unequally distributed in the five food samples examined. None of the aflatoxin types was detected in the groundnut, while the maize sample contained only AFG₁ and AFG₂. The bulk of the aflatoxin species were found in the bush mango and red pepper samples. This variation in occurrence of the various aflatoxin types may be attributable to differences in the chemical composition of the food commodities used in the study. Such differences in food chemical composition leading to variation in aflatoxin content have been reported by Schatzki and Ong (2001). The food chemicals could interact with some environmental factors to bring about differences in the growth of microbes and in the subsequent formation of aflatoxins in the various commodities. These environmental factors include excessive moisture in the field, storage temperature, humidity, drought, variations in harvesting practices and insect infestations. These factors have been shown to determine the severity of mycotoxin contamination of foodstuffs (Hussein & Brasel, 2001).

It is however surprising that the groundnut samples used in this study did not show any detectable levels of the aflatoxin types assayed, especially AFB₁. A near similar observation was also made on the maize sample, which showed detectable levels of contamination only with the AFG₁ and AFG₂. Studies in other locations within and outside Nigeria have shown high levels of aflatoxin contamination in groundnut, the commonest being aflatoxin B₁ (Williams et al., 2004; Jimoh & Kolapo, 2008; Kamika, 2012; Oranusi & Olarewaju, 2013). Also, it has been demonstrated that maize is one of the most commonly contaminated foods with aflatoxins (Williams et al., 2004; Muthomi, Njenga, Gathumbi, & Cheminingwa, 2009; Oranusi & Olarewaju, 2013). For instance in Nairobi, Kenya, an outbreak of aflatoxicosis resulting in the death of 125 of the 317 (39.4%) cases reported was traced to

the contamination of maize and its products from retail shops (Muture & Ogana, 2005; Muthomi et al., 2009). However, Scussel and Baratto (1994) in their studies on the aflatoxin levels in grains including maize from Brazil did not find any detectable levels of the four aflatoxin types assayed in this work. These researchers attributed their findings to improved harvesting and storage practices, as well as weather during that particular year. It is most probable that these factors were responsible for the results obtained in this work more so, since the various foodstuffs were collected in January, which in Calabar – Nigeria is a period of dry season when the relative humidity is generally low.

The high levels of contamination of bush mango with aflatoxins B₁, B₂, G₁ and G₂ observed in the present study agrees with the findings by Adebayo-Tayo et al. (2006) in some samples of bush mango (*Irvingia gabonensis*) examined in some markets in Akwa Ibom State, Nigeria. This was attributed to a number of factors including possible infection of the bush mango during cracking/ de-shelling to extract the cotyledons, drying, sorting as well as transportation. Akwa Ibom State shares a common boundary with Cross River State where this study took place, with similar climatic and socio-cultural features. It is therefore possible that all or some of these factors were responsible for the high levels of the aflatoxin types found in the bush mango samples in the present study.

Melon seed has also been reported (Bankole & Joda, 2004) to contain a level of AFB₁ similar to the results of this study. Also, this study obtained some levels of the four aflatoxin types in the red pepper. Studies by Aydin et al. (2007) had found aflatoxin B₁ in red pepper even though the levels in some of the samples collected were below the maximum tolerable limit. This could possibly be as a result of differences in the chemical composition of the food substrates. It is possible that different aflatoxin types have preference for certain foods depending on their chemical composition.

5. Conclusions

The results of our investigation demonstrate that the aflatoxigenic fungus (*Aspergillus*) aided by moisture and other factors is a common agent of contamination of foodstuffs marketed in Calabar. However, the levels of total aflatoxin in most of the food commodities are within acceptable limits and not all of them contain the B₁, B₂, G₁ or G₂ aflatoxins. The total and individual aflatoxin levels in the bush mango were generally above the maximum allowable limits of NAFDAC. Reduction of aflatoxin levels in food ingredients in Calabar and indeed Nigeria especially in bush mango should be a public health priority.

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