Aflatoxin B₁ Contamination of Some Edible Grains Marketed in Nigeria

S. A. ODOEMELAM* and C. I. OSU

Department of Chemistry,
Michael Okpara University of Agriculture,
Umudike, P. M. B. 7267, Umuahia, Nigeria.
saodoemelam@yahoo.com

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Abstract: This paper presents an assessment of the aflatoxin B₁ contamination of some food grains (wheat, millet, Guinea corn, breadfruit and groundnut) from major markets in the Niger Delta region of Nigeria. The concentrations of aflatoxin B₁ obtained ranged from 17.01-20.53 $\mu\text{g kg}^{-1}$ for wheat, 34.00– 40.30 $\mu\text{g kg}^{-1}$ for millet, 27.22-36.13 $\mu\text{g kg}^{-1}$ for guinea corn, 40.06-48.59 $\mu\text{g kg}^{-1}$ for breadfruit and 74.03-82.12 $\mu\text{g kg}^{-1}$ for groundnut. Aflatoxin B₁ was detected in all the samples. There were significant differences ($p < 0.01$) in the levels of aflatoxin B₁ determined in all the samples and the toxin contamination was not restricted to any particular section of the region. The presence of aflatoxins in grains constitutes a serious health hazard to both human beings and animals because of their toxic and carcinogenic property.

Keywords: Aflatoxin, Grains, Contamination, Nigeria,

Introduction

Aflatoxins are produced in nature by *Aspergillus flavus* and *Aspergillus parasiticus*^{1, 2}. The organisms are ubiquitously found in air and soil and they infest both living and dead plants and animals. Aflatoxin may be categorized as secondary metabolites and their production is influenced by factors such as catabolic activity, reduced coenzymes level and metal ions^{3, 4}. *A. flavus* and *A. parasiticus* have been found during growth, harvest and storage of different foods and feeds⁵. According to Farombi⁶ the fungi contaminate a large number of dietary staples and agricultural products such as rice, corn, cassava, peanuts and spices. Consequently, human beings and animals are exposed to aflatoxin by consuming contaminated food. According to Bankole and Adebajo⁷, in many parts of Africa, the need to eat outweighs other considerations such as food safety, thereby making food-borne intoxications to be a serious problem.

Awareness of the potential danger posed by aflatoxins contamination of foodstuffs has been on the increase in recent times. Aflatoxins are generally considered a potential hazard to public health due to their toxicity, mutagenicity, tetratogenicity and carcinogenicity⁸⁻¹⁰. Thus the presence of aflatoxin in feeding stock has far reaching deleterious effects on animal and human health. Dietary exposure to aflatoxin B₁ has been identified as a major etiological risk factor for the development of hepatocellular carcinoma^{6,11}. Lanyasunya *et al.*,¹² have reported that an outbreak of aflatoxin poisoning in Kenya (January – July 2004) resulted in 125 recognized deaths and hospitalization of over 300 others. As a result, the presence of these toxins in agricultural commodities has a potential negative impact on the economies of the affected regions especially in the developing countries where there are inadequate harvest and post-harvest techniques for the prevention of mould growth. Hence the contaminated commodities suffer serious discrimination and, in most cases, utter prohibition especially in international trade. Recently, Russell and Paterson¹³ reported that chilli production in Pakistan may be heavily constrained by aflatoxin contamination.

The increased concern in the microbiological safety of food has led to the use of several methods, including artificial preservatives to prevent or reduce the incidence of mycotoxins in food commodities. Ogunsanwo *et al.*,¹⁴ reported that mere roasting at 150°C for 30 min reduced the aflatoxin content of Nigerian peanut by 70% while Onilude *et al.*,² reported that certain lactic acid bacteria isolates from indigenously fermented cereal gruels inhibited the growth of aflatoxigenic mould.

Aflatoxins are found in many countries of the world, especially in tropical and subtropical regions where the warm and humid weather provides optimal conditions for the growth of aflatoxigenic moulds. The optimum temperature for the growth of the moulds is 24 – 35°C and equilibrium relative humidity of above 70%⁶⁻¹⁶. Thus food and feed crops grown under tropical and subtropical conditions are more prone to aflatoxin contamination than those in temperate regions. Nigeria, being a tropical country, her conditions of temperature and humidity are favourable for growth of the moulds and for the production of the toxin.

Ingestion of aflatoxins by farm animals consuming contaminated feeds leads to substantial loss of productivity and degradation of meat quality^{17,18}. Human exposure to aflatoxins can result directly from ingestion of contaminated foods or indirectly from consumption of foods from animals previously exposed to aflatoxin in feeds¹⁵. Epidemiological studies regarding the hazardous nature of aflatoxins as potent carcinogens have shown that they cause liver cirrhosis and induce tumors in populations exposed to the toxin from contaminated foods^{2, 19-22}.

Four different aflatoxins, B₁, B₂, G₁ and G₂ have been identified with B₁ being the most toxic¹². Therefore, the present study was undertaken to determine the post-harvest levels of aflatoxin B₁ contamination in millet, wheat, Guinea corn, breadfruit and groundnut marketed in the Niger Delta region of Nigeria.

Experimental

Collection of samples

The grains studied were purchased from markets in the Niger Delta region (Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Imo, Ondo and Rivers States) of Nigeria. Samples were collected from November 2004 to March 2005. A portion of each sample was weighed (500 g), ground to fine powder using electric blender (Philips, HR 2815, Japan) and stored in air tight bottles at 4 °C till required for analysis.

Aflatoxin analysis

A portion of each ground sample was dispensed into a 500 mL Erlenmeyer flask containing 100 mL of *n*-hexane-petroleum ether (1:10) and kept at room temperature overnight to extract fat. After filtration the samples were treated with 100 mL of 50% aqueous acetone and shaken for 2 h on a mechanical flask shaker (Griffin and George Ltd.) before filtering through a two-fold muslin cloth. Aflatoxins were extracted into 20 mL chloroform. The chloroform layers were drawn off into 250 mL round bottomed flasks and evaporated to dryness using a rotary evaporator. The extracted aflatoxins were separated by thin-layer chromatography (TLC) using 20 x 20 cm plates coated with silica gel (0.5 mm thickness) and developed in 3% methanolic chloroform solution. Aflatoxin B₁ was identified by TLC against standard (aflatoxin B₁) Rf value and fluorescent properties under uv light. The concentration of aflatoxin B₁ was determined spectrophotometrically ($\lambda=363$ nm, $\gamma=22,000$) by the method of Opadokun²³ using the equation:

$$A = \gamma c,$$

where *A* is Absorbance, γ is molar extinction coefficient and *c* is concentration.

Results and Discussion

The levels of aflatoxin B₁ ($\mu\text{g kg}^{-1}$) in the grains investigated are presented in Table 1 while the mean concentrations are shown in Table 2. The results reveal moderate levels of aflatoxin B₁ (17.10 – 82.12 $\mu\text{g kg}^{-1}$) in all the samples examined. The lowest aflatoxin B₁ concentrations were recorded for wheat while groundnut had the highest. The results also show that the locations where the samples were collected had a significant effect on aflatoxin B₁ levels ($p < 0.05$) except for wheat and Guinea corn for which aflatoxin B₁ concentration did not vary significantly with some locations ($p < 0.01$).

Table 1. Levels of aflatoxin B₁ ($\mu\text{g kg}^{-1}$)* in food grains from markets in Nigeria

Location	Wheat	Millet	Guineacorn	Breadfruit	Groundnut
Abia	18.41 ^{uv} $\pm$ 0.39	35.00 ^{NQ} $\pm$ 2.25	32.76 ^{QR} $\pm$ 0.70	45.17 ^{HI} $\pm$ 0.17	79.56 ^{BD} $\pm$ 2.44
Akwa Ibom	19.50 ^{uv} $\pm$ 0.50	39.00 ^{LM} $\pm$ 1.00	34.50 ^{OQ} $\pm$ 1.11	40.06 ^{KL} $\pm$ 0.06	75.47 ^{EF} $\pm$ 1.29
Bayelsa	20.53 ^u $\pm$ 0.66	36.54 ^{NO} $\pm$ 0.89	27.40 ^T $\pm$ 0.20	42.31 ^{JK} $\pm$ 1.96	82.12 ^A $\pm$ 0.07
Cross River	17.10 ^v $\pm$ 0.90	37.36 ^{MN} $\pm$ 0.56	36.13 ^{NP} $\pm$ 0.25	44.31 ^{IJ} $\pm$ 0.11	76.68 ^E $\pm$ 1.07
Delta	18.80 ^{uv} $\pm$ 0.20	40.00 ^{KL} $\pm$ 2.00	29.00 ST $\pm$ 1.25	48.29 ^G $\pm$ 0.45	79.81 ^{BC} $\pm$ 0.26
Edo	19.60 ^{uv} $\pm$ 0.30	34.00 ^{PQ} $\pm$ 2.00	28.01 ^T $\pm$ 1.05	46.62 ^{G-I} $\pm$ 0.57	74.03 ^F $\pm$ 1.90
Imo	18.45 ^{uv} $\pm$ 0.24	36.33 ^{N-P} $\pm$ 1.04	28.72 ^T $\pm$ 0.72	45.76 ^{HI} $\pm$ 0.04	77.65 ^{CE} $\pm$ 1.34
Ondo	19.00 ^{uv} $\pm$ 0.20	39.19 ^{LM} $\pm$ 1.01	31.00 ^{RS} $\pm$ 2.00	48.59 ^G $\pm$ 0.64	77.37 ^{DE} $\pm$ 1.17
Rivers	19.59 ^{uv} $\pm$ 2.10	40.30 ^{KL} $\pm$ 1.13	27.22 ^T $\pm$ 0.22	47.18 ^{GH} $\pm$ 0.23	81.22 ^{AB} $\pm$ 1.14

*Results are mean of triplicate determinations $\pm$ S.D (Standard deviation) Results with the same letter(s) are not significantly different ($P \leq 0.01$ and $P \leq 0.05$).

Table 2. Mean Concentration of aflatoxin B₁ ($\mu\text{g kg}^{-1}$) in food grains from markets in Nigeria

Grains	Mean concentration*
Wheat	19.00 ^E $\pm$ 1.67
Millet	37.52 ^C $\pm$ 2.52
Guinea corn	30.53 ^D $\pm$ 3.37
Breadfruit	45.37 ^B $\pm$ 2.76
Groundnut	78.21 ^A $\pm$ 2.92

*Results with different letters (A-E) are significantly different at $p < 0.05$

It seems that differences in harvest and storage conditions as well as the agricultural practices (in each location) also influenced the level of aflatoxin contamination of the commodities. Three environmental factors (temperature, relative humidity and amount of rainfall) influence the production of aflatoxin in the field and during storage. Studies done on the effect of environmental conditions on aflatoxin contamination of corn showed that, when the conditions were favourable, the occurrence of aflatoxin was highly related to these factors⁵. Temperature is an important factor for growth of *Aspergillus flavus*²⁴. In a study on the effect of temperature on aflatoxin production, Viquez *et al.*,⁵ observed that aflatoxin level was significantly ($p<0.05$) affected by temperature. The study area has optimal temperature, humidity and light for the growth of aflatoxigenic moulds. This may be responsible for the detection of aflatoxin B₁ in all the samples.

Aflatoxin B₁ concentrations in the wheat samples analyzed ranged from 17.10 – 20.53 $\mu\text{g kg}^{-1}$ with a mean value of 19.00 $\mu\text{g kg}^{-1}$. Wheat is the major source of flour for baked foods. Considering the FDA tolerance level for total aflatoxin in food for human consumption (Table 3), the aflatoxin B₁ levels reported for wheat signal a note of caution against prolonged consumption of the commodity in view of the health hazard associated with aflatoxins.

Table 3. FDA tolerance levels for total aflatoxin

Item	Tolerance level, $\mu\text{g kg}^{-1}$
Food for human consumption	20
Food for beef cattle and poultry	300
Feed for swine	200
Feed for breeding livestock	100
Feed for dairy cattle	20
Milk	0.5

Source: Ref. 15

Millet is a major part of staple food in northern parts of Nigeria. Mishra and Daradhiya (1991) reported aflatoxin levels ranging between 17 – 2110 $\mu\text{g kg}^{-1}$ in millet (Table 4). In this study, levels of aflatoxin found in millet ranged from 34.00 – 40.30 $\mu\text{g kg}^{-1}$ with a mean value of 37.52 ± 2.52 $\mu\text{g kg}^{-1}$. Millet from Akwa Ibom had approximately the same level of aflatoxin B₁ with millet from Ondo State. Similarly the concentration of aflatoxin B₁ in millet from Delta State did not differ significantly ($p<0.01$) with that from Rivers State. Significant differences ($p<0.05$) were observed in aflatoxin B₁ concentrations of the rest of the millet samples.

Guinea corn is another staple food in most parts of northern Nigeria. The levels of aflatoxin B₁ found in Guinea corn varied between 27.22 and 36.13 $\mu\text{g kg}^{-1}$ with a mean concentration of 30.53 ± 3.37 $\mu\text{g kg}^{-1}$. The levels aflatoxin B₁ obtained for Guinea corn from Abia, Akwa Ibom, Cross River, Delta, Ondo and Rivers States varied significantly ($p<0.05$) showing the effect of location and environmental factors. However, there was no significant difference ($p<0.01$) in the aflatoxin B₁ levels in the Guinea corn samples from the rest of the states.

The levels of aflatoxin B₁ in breadfruit ranged from 40.06 to 48.59 $\mu\text{g kg}^{-1}$. Most of the results obtained for breadfruit are significantly different ($p<0.05$) showing the effect of location and environmental factors on the production of aflatoxin B₁. Breadfruit is widely consumed in the eastern parts of Nigeria. Therefore, prolonged consumption of a large quantity of breadfruit may constitute a public health hazard.

The levels of aflatoxin B₁ found in the groundnut samples investigated ranged from 74.03 to 82.12 $\mu\text{g kg}^{-1}$ with a mean concentration of 78.21 ± 2.92 $\mu\text{g kg}^{-1}$. Park and Njapau²⁶

reported aflatoxin B₁ levels between 20 – 200 µg kg⁻¹ in peanut from Senegal and Argentina, while Singh *et al.*,²⁷ reported aflatoxin B₁ levels between 33 – 440 µg kg⁻¹ in peanut from India (Table 4). In two different studies conducted in Nigeria, McDonald²⁸ reported aflatoxin levels up to 2000 µg kg⁻¹ in peanut while Akano and Atanda²⁹ reported aflatoxin B₁ levels between 37 – 455 µg kg⁻¹ in groundnut cake (kulikuli). Although the levels of aflatoxin B₁ (74.03-82.12µg kg⁻¹) now being reported for groundnut is within the range reported elsewhere^{26,27}, differences between these results and that of the present investigation may be due to different geographical origin, seasonal variation of the samples tested and weather conditions. Among all the samples studied, groundnut had the highest level of aflatoxin B₁. The reason why the incidence of aflatoxin is more frequent in groundnut than in other agricultural commodities is not fully understood¹⁵.

Table 4. Occurrence of aflatoxin in some commodities in certain countries

Country	Commodity	Aflatoxin	Concentration, µg kg ⁻¹	Reference
Nigeria	Sorghum	B ₁	30.22-211.20	31
	Groundnut	-	up to 2000	28
	Peanut cake	B ₁	37-455	29
	Shelled melon	-	5-20	32
	Yam chips	-	4-186	7
	Maize	-	3-138	33
Ghana	Maize	-	20-355	34
	Maize dough	-	0.7-313	34
Botswana	Peanut butter	-	0.3-23	35
S. Africa	Sorghum	B ₁	0-25	36
Senegal	Groundnut	B ₁	20-200	26
India	Sorghum	B ₁	7-75	37
	Millet	B ₁	17-2110	25
	Groundnut	B ₁	33-440	27
	Pistachio nuts	-	15-259	38
Bangladesh	Groundnut	-	65	39
Turkey	Hazelnut Cream	B ₁	0.126->5	40
Argentina	Groundnut	B ₁	20-200	26
	Cotton seed	B ₁	20-200	26

Apart from serving as staple foods for many Nigerian homes, most of the grains investigated are used in compounding animal feeds. Most of the grains investigated contained aflatoxin B₁ above the 20 µg kg⁻¹ Nigerian tolerance level for aflatoxin B₁ in foods⁶. Mean levels of aflatoxin B₁ in the samples were also above the EEC³⁰ maximum permitted level of aflatoxin B₁ for certain animal feeds.

Conclusion

The results generally revealed the presence of aflatoxin B₁ in all the samples analysed. Groundnut had the highest level of aflatoxin B₁ compared to other foods studied. The grains reported here are contaminated with aflatoxin B₁ at levels that cannot be said to be totally toxicologically 'safe'. Prolonged consumption of large quantities of the grains investigated may lead to aflatoxin related intoxications in livestock and human beings. Therefore, there is need to prevent aflatoxin contamination in the grains by maintenance of adequate and rapid drying of the grains to a water content at which the growth of aflatoxigenic fungi can be reduced to the barest minimum.

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