

Total Aflatoxin Contamination of Wheat, Groundnut and Their Products Sold in Three Markets within Port-Harcourt Metropolis, Nigeria

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Abstract

Aflatoxins are a group of toxic and carcinogenic secondary metabolites of fungal origin produced by strains of *Aspergillus* genera. This study presents an assessment of total aflatoxin contamination of wheat, groundnut and their products marketed within Port Harcourt metropolis. Total aflatoxin concentration was determined in all the samples using AgraQuant aflatoxin ELISA test kit and quantified using the Stat fax ELISA reader. The mean total aflatoxin concentration ranged from 1.73 ± 0.09 $\mu\text{g}/\text{kg}$ to 37.23 ± 3.66 $\mu\text{g}/\text{kg}$ amongst all samples. Amongst the wheat and wheat products, bread samples from mile one market had the highest value of 1.73 ± 0.09 $\mu\text{g}/\text{kg}$ while raw wheatmeal (branded) samples from oil mill market had the lowest value of 0.70 ± 0.06 $\mu\text{g}/\text{kg}$; amongst the groundnut and groundnut product group, defatted groundnut cake (Kuli-kuli) samples from mile one market had the highest value of 37.23 ± 3.66 $\mu\text{g}/\text{kg}$ while boiled groundnut samples had the lowest value of 0.80 ± 0.12 $\mu\text{g}/\text{kg}$. With the exception of defatted groundnut cake (kuli-kuli) and groundnut paste, all the other samples analyzed were found to be within the acceptable limits as set by NAFDAC of 10 $\mu\text{g}/\text{kg}$ for raw food (yet to be cooked) and 4 $\mu\text{g}/\text{kg}$ for cooked/ready-to-eat food.

Keywords: aflatoxin; ELISA; carcinogenic; *Aspergillus flavus*.

INTRODUCTION

Food is a very important aspect of human life and its safety is a priority if the health of the people is to be guaranteed. Mycotoxins, especially aflatoxins, have received considerable attention due to their significance in agricultural loss and human health. The Food and Agricultural Organisation (FAO) of the United Nations, has estimated that up to 25% of the world's food crops have been estimated to be significantly contaminated with mycotoxins especially aflatoxins (WHO, 1999). Aflatoxins are a group of toxic and carcinogenic secondary metabolites of fungal origin. They are produced by strains of *Aspergillus flavus*, *A. parasiticus* and, in rare cases, *A. nominus* and *A. pseudotamari* (Mazaheri, 2009). Humans are exposed to aflatoxin contamination directly (by consuming contaminated food and food products) or indirectly (by consumption of meat of animals fed with contaminated feeds). Foods that are commonly affected include all nuts especially groundnuts, tree nuts such as pistachio and brazil nuts, cottonseed, copra, rice, maize, wheat, sorghum, pulses, figs, and oilseed cakes. There are at least sixteen (16) different types of aflatoxins produced in nature but only six (6) of these are of analytical interest (FSSAI, 2012). Four occur in foods and two as metabolites in the milk of animals who have been fed contaminated feed. Aflatoxins B₁, B₂, G₁ and G₂ refers to toxins which fluoresce blue (B) or green (G) under ultraviolet light and are separable by thin layer chromatography (FAO, 1986). Amongst the various types of aflatoxins, aflatoxin B₁ (AFB₁) is the most lethal metabolite and is a known human carcinogen (WHO, 2006).

Studies have shown the hazardous nature of aflatoxins both to humans and animals. Hepatocellular carcinoma (liver cancer) is the most common disease that results from the consumption of a high amount of aflatoxins (William *et al.*, 2004), and WHO (2010) has said that it is the third leading cause of cancer each year. The greatest risk of health impact lies within developing countries located in the tropical regions, which rely on these commodities as their staple food source. Food insufficiency and lack of diversity substantially contribute to the susceptibility of individuals and communities to aflatoxins. No animal species is immune to the acute toxic effects of aflatoxins; however, adult humans have a high tolerance for aflatoxin exposure and rarely succumb to acute aflatoxicosis (Williams *et al.*, 2004). Aflatoxicosis is the disease associated with the ingestion of aflatoxins. Outbreaks of aflatoxicosis have been reported in Kenya, India and Thailand, but none has been reported so far in Nigeria. The outbreak which occurred in Kenya is one of the largest and most acute outbreaks of aflatoxicosis ever documented (CAST, 2004), with 317 cases reported and 125 deaths recorded (CDC, 2005). Several factors contribute to the incidence of aflatoxin contamination in tropical countries including Nigeria. These factors include favourable climate, poor conditions of production and storage of agricultural commodities, soil type, humidity and inadequate drying of food crops before storage. It has been reported that storage increases the rate of fungal contamination as well as the aflatoxin concentration in both raw and processed food (Adebajo *et al.*, 1994). Aflatoxin contamination poses a potential threat to food safety in Nigeria because in most parts of the country and amongst the greater number of indigent populace, food safety is not a concern as they are often only interested in eating food notwithstanding any health implications. Thus, as aflatoxin is epidemiologically implicated as a

carcinogen in humans and an environmental contaminant which is widespread in nature, its possible chronic toxicity is therefore of greater concern than acute toxicity. The presence of mycotoxins in our food systems and tissues has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic (Onyemelukwe *et al.*, 2012). They are also capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular, pulmonary systems and intestinal tract (Bhat and Vasanthi, 2003). Aflatoxins are of great concern especially in children and childhood because there is an increased pre-five mortality and reduced life expectancy (Miller, 1996).

MATERIALS AND METHODS

Study area

The study was carried within Port-Harcourt metropolis in south-south Nigeria. Samples were collected from three markets including Mile one market, Creek road market and Oil mill market.

Materials/equipment

Tween water, methanol analar, distilled water, de-ionized water, filter paper, microtitre wells, adsorbent towel, measuring cylinder, multichannel pipette. Equipment used includes AgraQuant® aflatoxin ELISA test kit, Stat fax ELISA reader, Sartorius automated moisture analyzer, Mettler-Toledo weighing balance and Kenwood electric miller,

Extraction and quantification of aflatoxin

The aflatoxin in the samples was measured using the AgraQuant® aflatoxin test kit by Romers laboratories, USA. The AgraQuant® kit used for the assay is a complete set with 6 standards, 48 antibody-coated microwells, 48 colour coded dilution microwells, 8-channel pipette, substrate, conjugate, and stop solution. The ELISA technique and AgraQuant® kit has been reported to be very accurate in terms of sensitivity and accuracy. 20g of each sample were ground with the Kenwood electric mill to a fine powder that will pass 20-mesh sieve and thoroughly mixed together. The mill was properly cleaned after milling each sample before proceeding to the next sample. The powder raw samples and wet samples did not need further preparation hence 5g of each sample was taken for aflatoxin extraction. The aflatoxin content was determined by using enzyme-linked immunoassay (ELISA) technique. The principle involves the use of methanol/tween water solution to extract aflatoxin from finely ground samples, dilution using distilled water followed by filtration and test using immunoassay technique in microtitre wells. Aflatoxin-HRP enzyme conjugate is pipette into the test wells followed by addition of calibrators or sample extracts into appropriate test wells. Aflatoxin antibody is then pipette into the test wells to initiate the reaction. This is then incubated for 10-15 minutes for reaction to take place. During this time, aflatoxins from the sample and aflatoxin-HRP enzyme conjugate compete for binding to aflatoxin antibody which in turn binds to the test wells. Following 10 minutes incubation, the contents of the wells are discarded and the wells washed to remove any unbound toxin or enzyme-labelled toxin. A clear substrate is then added to the wells and any unbound enzyme toxin conjugate causes a colour change to blue. Following 15 minutes incubation, the reaction is stopped with a stop solution and the colour changes to yellow. This is then read off in the stat fast ELISA reader set for Aflatoxin by following manufacturer's instructions. The results are then printed out in a slip showing the various concentration of aflatoxin in all the samples.

STATISTICAL ANALYSIS

All the values were expressed as mean \pm standard error. The data obtained from the analysis was subjected to statistical analysis of variance (ANOVA) using the SPSS® statistical package (version 20) from IBM® Inc, USA. The means were separated and compared at 0.05 level of significance.

RESULTS

The results obtained from the study are shown in the tables below:

Table 1: Total Aflatoxin Concentration ($\mu\text{g}/\text{Kg}$) of Wheat and its Products

| SAMPLE | MARKETS | | |
|------------------------------|------------------------------|------------------------------|--------------------------------|
| | MILE 1 | CREEKROAD | OIL MILL |
| Raw dried wheat grains | 1.00 \pm 0.06 ^a | 0.90 \pm 0.06 ^a | 1.17 \pm 0.12 ^a |
| Flour | 0.80 \pm 0.06 ^a | 0.87 \pm 0.03 ^a | 1.67 \pm 0.12 ^b |
| Bread | 1.73 \pm 0.09 ^a | 1.40 \pm 0.12 ^a | 0.80 \pm 0.12 ^b |
| Wheatmeal(branded, raw) | 1.0 \pm 0.11 ^a | 1.17 \pm 0.12 ^a | 0.70 \pm 0.06 ^{b,c} |
| Wheatmeal(unbranded, raw) | 0.93 \pm 0.03 ^a | 1.07 \pm 0.12 ^a | 0.97 \pm 0.12 ^a |
| Unbranded wheatmeal (cooked) | 0.73 \pm 0.03 ^a | 0.87 \pm 0.03 ^a | 1.10 \pm 0.15 ^a |
| Branded wheatmeal(cooked) | 0.83 \pm 0.09 ^a | 0.87 \pm 0.12 ^a | 1.83 \pm 0.19 ^b |

Values are presented as mean \pm standard error of triplicate determination

Values in each row with different superscript letter differ significantly at level $p < 0.05$.

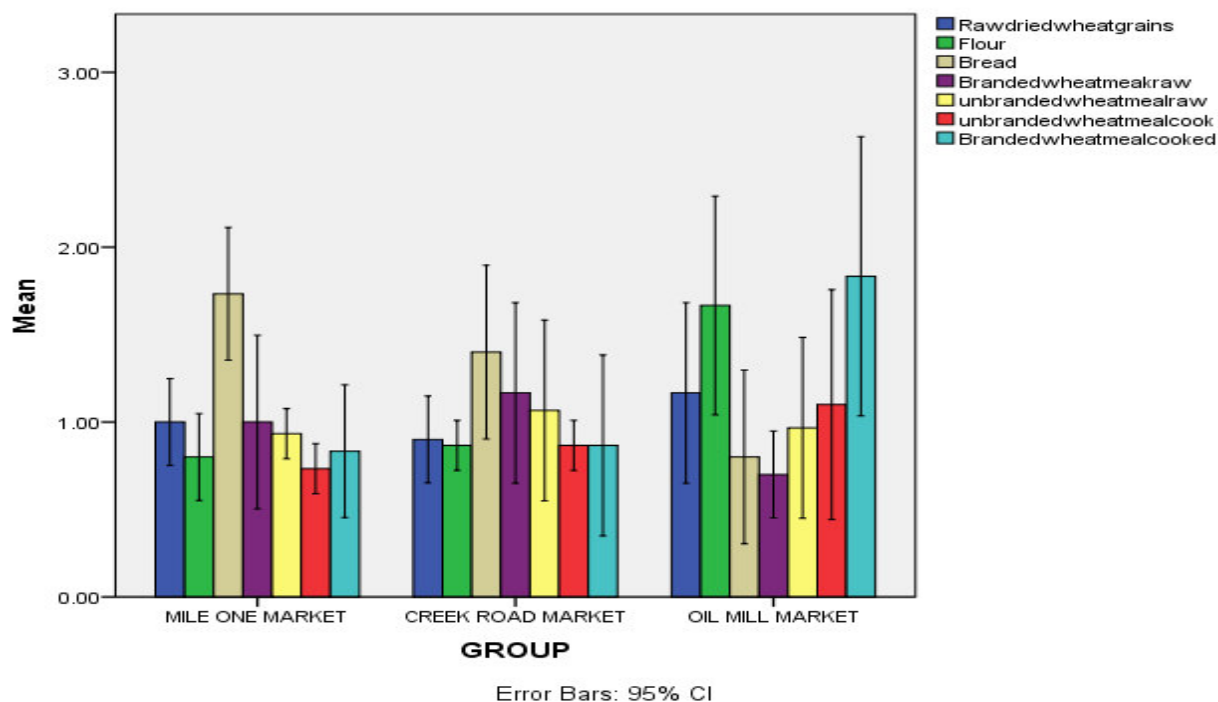


Figure 1: Bar chart presentation of aflatoxin concentration in Wheat and wheat products marketed in three markets in Port Harcourt metropolis

Table 2: Total Aflatoxin Concentration (µg/Kg) of Groundnut and its Products

| SAMPLE | MARKETS | | |
|-------------------------------------|-------------------------|-------------------------|-------------------------|
| | MILE ONE | CREEKROAD | OIL MILL |
| Raw groundnut seeds | 1.27±0.12 ^a | 1.50±0.12 ^a | 1.13±0.09 ^a |
| Boiled groundnut seeds | 0.80±0.12 ^a | 1.40±0.31 ^a | 0.73±0.09 ^a |
| Roasted groundnut seeds | 1.30±0.12 ^a | 1.70±0.10 ^a | 1.47±0.31 ^a |
| Defatted groundnut cake (Kuli-kuli) | 37.23±3.66 ^a | 35.43±1.45 ^a | 34.73±2.89 ^a |
| Groundnut paste | 16.23±1.19 ^a | 17.87±0.24 ^a | 15.40±0.32 ^a |

Values are presented as mean ± standard error of triplicate determination

Values in each row with different superscript letter differ significantly at level $p < 0.05$

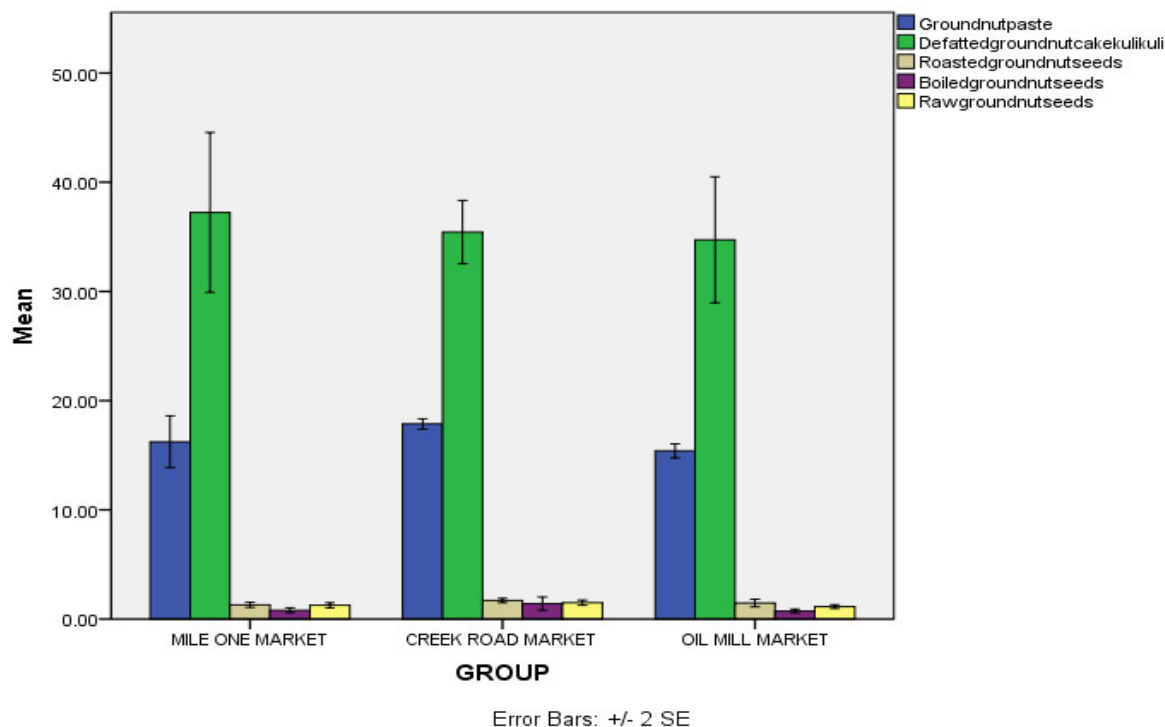


Figure 2: Bar chart presentation of aflatoxin concentration in groundnut and its products marketed in three markets in Port Harcourt metropolis

DISCUSSION

Incidence of aflatoxin in high concentration in groundnut seeds has been reported by various researchers (Makun, 2013). The results of this study are however in agreement with the reports of Abalaka and Elegbede, (1982) and Opadokun, (1992). The findings of this study on kuli-kuli and groundnut paste were in agreement with the works of Akano and Atanda, (1990) and Ezekiel *et. al.*, (2012). From the results of this study, it is evident that moisture content and visible mouldy nature of foods products are very important determinants of aflatoxin content. The aflatoxin content of several of the samples was significantly low because water is very important for the survival of the fungus that produces aflatoxin. The only exceptions were seen in the kuli-kuli and groundnut paste samples in which despite the low moisture content, the total aflatoxin levels were significantly higher than in all other sample. This may not be unconnected with the hygienic status of handlers of these food products. For the samples analyzed, it could also be that the low aflatoxin levels recorded is a positive indicator of the awareness of farmers on combating aflatoxin.

CONCLUSION

From the study it can be concluded that the total aflatoxin content of groundnut and its products are significantly higher when compared to those of wheat and wheat products. With the exception of groundnut cake and groundnut paste, all other samples analyzed had aflatoxin content within safe limits with respect to NAFDAC guidelines.

RECOMMENDATION

From the findings of this work, it is observed that there is limited availability of data on total aflatoxin in cooked wheatmeal hence further research should be carried out in this area. Also routine analysis of groundnut cake and groundnut paste offered for sale should be done to monitor the level of exposure of unsuspecting Nigerians who are mostly in the lower class and ignorant of the occurrence of such toxins in the foods they consume.

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