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Aflatoxins composition of maize (*Zea mays* L.), guinea corn (*Sorghum bicolor* L.) cold paps and peanut (*Arachis hypogea* L.) butter in Nsukka, Nigeria

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Abstract

Contamination of foodstuffs by aflatoxins is a serious threat to food safety and security in most parts of the world and there is need to sensitize consumers on the aflatoxin contents of the foods they consume. This study was carried out to ascertain levels of aflatoxin contamination in some locally prepared and marketed cold paps of maize and guinea corn as well as peanut butter in Nsukka, Nigeria. Aflatoxins in the samples bought from three different markets in Nsukka, Enugu State, Nigeria were analyzed in triplicates using thin layer chromatography and spectrophotometric methods. Results showed that aflatoxin B_1 and B_2 were detected in all the food samples with their concentrations ranging from 1.48 ± 0.24 to 2.31 ± 0.64 µg/kg and 1.42 ± 0.17 to 2.17 ± 0.83 µg/kg respectively. Aflatoxins B_1 and B_2 concentrations were statistically different ($p \leq 0.05$) in all sampled food products. Aflatoxin B_1 differed significantly ($p \leq 0.05$) among locations. The total aflatoxin contents of sampled foods ranged from 8.9 ± 0.9 to 49.1 ± 9.0 µg/kg and differed significantly ($p \leq 0.05$) among samples and locations. Unsafe levels of mainly the total aflatoxins were associated with cereal cold paps and peanut butter sold in the study area. Though concentrations of aflatoxins (B_1 and B_2) in these foods were within recommended tolerable limits, that of total aflatoxin contents were above the tolerable limits (mostly in peanut butter) and are potential threats to consumers.

Key words: Aflatoxins, cold paps, guinea corn, maize, peanut butter

1. Introduction

Aflatoxins are secondary metabolites of some fungi produced on their hosts/substrates when conditions are favourable [1]. Aflatoxins came to the lime light in the 60's with the outbreak of Turkey X-Disease in Great Britain [2]. The disease killed thousands of turkey birds in England and *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare were associated with it as the causal agents. Also, *Aspergillus nominus* and *A. tamari* have afterwards been reported to have the capacity to produce the toxins [3]. Since then over thirty aflatoxins have been discovered, the most popular of which are Aflatoxin B_1, B_2, G_1 and G_2 [4]. Aflatoxin production/synthesis by the associated fungi have been attributed to some factors which include, temperature, moisture content, relative humidity, substrate composition and the presence of competing organisms [5, 6, 7]. They are known to be teratogenic, mutagenic, immunosuppressive and hepatotoxic [8, 9, 10]. Crops could get infected with aflatoxin producing fungi (e.g. *Aspergillus* species) in the field, in storage, in transit and in the processing facilities though aflatoxin production is encouraged by high temperature, moisture and heavy rains [9, 11, 12]. Aflatoxins have an alarming prevalence of 43.5% in African countries [12]. In Nigeria, aflatoxins have been detected in locally produced beverages [13], maize [14], groundnut [15], yam flour, cassava flour, processed cassava (garri), beans and rice [16]. The death of over 1,000 six-week old birds in the University of Nigeria, Nsukka was associated with feed contaminated with aflatoxin B_1 [17]. When aflatoxin B_1 is ingested by cow, it is transformed into hydroxylated products, aflatoxins M_1 and M_2 and such aflatoxins are secreted during milk pasteurization, storage and preparation of various dairy products [18]. Aflatoxins attract worldwide attention because they are thermostable and are often found in final products that passed through

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conventional and technological food processing procedures [4, 19].

Maize (*Zea mays* L.) and guinea corn (*Sorghum bicolor* L.), constitute the most common cereal grains that are grown and eaten in Nigeria [20]. Groundnut or peanut (*Arachis hypogea* L.) on the other hand, is an important grain legume in Nigeria, serving as food for man and livestock as well as an industrial raw material [21]. Maize and guinea corn are eaten in many ways, sometimes as pastes, roasts, semiliquid foods/paps, porridges and pottages. They possess fermentable carbohydrates and proteins in good quantity [22]. They may also be milled and further processed into flour, starch, corn oil, semiliquid foods, breakfast meals and dinner cakes. Locally, they are used to prepare *Tuwo, Akamu, Eko, Burukutu, Dawa* and *Ogi* [23]. In Nsukka, Nigeria, *Akamu* either from maize or guinea corn are consumed by majority of the people as breakfast meals. They are also used as weaning/complementary foods for children. In Nigerian markets, these foods are usually openly displayed for sales.

Groundnut is commonly eaten as paste or butter, cakes or roasted. Groundnut paste (also called peanut butter or *Ose oji* in South Eastern Nigeria, is a cohesive food product prepared from shelled groundnuts by grinding roasted kernels to which salt, pepper and *ehuru* spice (*Monodora myristica*) are added as a seasoning and flavouring agent. It is a common household product that is conventionally eaten with garden eggs (*Solanum melongena*) and kola nuts (*Cola acuminata)*, both in traditional ceremonial settings and domestic uses in Nsukka [24]. These products are very important in the domestic, social and nutritional lives of people in Nigeria and unfortunately there is little or no information on their aflatoxin contents. Therefore, the present work was undertaken to assess the composition of aflatoxin B_1, B_2 in cold paps produced from maize and sorghum, and peanut butter marketed in Nsukka, Nigeria.

2. Materials and methods

2.1. Collection of food samples

The maize cold pap *(Akamu*), guinea corn cold pap (*Dawa*) and groundnut butter (*Ose oji*) samples used in this study were purchased from three major markets in Nsukka area: Ogige main market in Nsukka Local Government Area, Nkwo Ibagwa Market in Igbo-Eze South Local Government Area and Orie-Orba Market in Udenu Local Government Area all in Enugu State, Nigeria. Samples were obtained with the aid of sterile polythene bags, labelled accordingly and transported to the laboratory for analysis. Each sample was collected from three different markets. A control standard for these food products was prepared in the laboratory using aseptic techniques [23]. Potassium dichromate (0.29 g) was dissolved in 50 mL of 0.009 M sulphuric acid. One part of the mixture and 10 parts of sulphuric acid was mixed in 10 different tubes and the absorbance was read at 350 nm [25].

2.2. Extraction of aflatoxins from food samples

The AOAC procedure [25] was adopted with some modifications for the purification, resolution and estimation of aflatoxins in the sampled food products. Ten grams of each sample was weighed into 100 mL corkable plastic containers and labelled accordingly. Twenty five mL of methanol-water (5.5 ml methanol + 4.5 mL water) mixture was added to each of the sample. Ten mL of n-hexane was also added to the mixture, which was shaken thoroughly for 1 hr. The resultant mixture was decanted into centrifuge tubes and labelled accordingly. The above procedure was repeated but in addition, 0.5 g of sodium chloride (NaCl) was added, and the mixture was shaken for 10 min and decanted into the same centrifuge tubes. Then the centrifuge tubes were centrifuged for 5 min at 3000 rpm. The aqueous methanolic phase (middle layer) (6 mL) was extracted with the aid of syringe into another set of centrifuge tubes, and chloroform (6 mL) was added. The chloroform extract was shaken for 1 min and centrifuged for 5 min at 3000 rpm. The layers separated and the bottom chloroform layer (2 mL) was drained into test tubes and also labelled accordingly. The test tubes were loaded into a beaker of water and evaporated to dryness or as soon as condensing vapour are no longer visible on beaker tip under gentle heating. The residue in each test tube was re-dissolved and reconstituted in 2 mL benzene-acetonitrile (9.8 mL benzene + 0.2 mL acetonitrile).

2.3. Thin layer chromatography of the extract

Pre-coated silica gel TLC plates of size 20 cm × 20 cm × 0.15 cm were used. The pre-coated silica gel TLC plates were first activated by heating in oven for 1 hr at 100 °C and finally allowed to cool at room temperature in desiccators [25]. The plates were spotted with the samples using micropipettes and transferred into a chromatographic tank (developing tank) containing the solvent (chloroform:acetone:propanol, 85:15:2.5 v/v). The tank was sealed with Vaseline, covered and the plates were left to develop to the solvent stop line. The plates were removed from the tank when they developed to the solvent stop line and left to dry at room temperature. The aflatoxins were detected using UV lamp (365 nm). Reference standards (Sigma-Aldrich, Germany) were prepared by dissolving 1 mg of aflatoxin in 100 mL of toluene + acetonitrile (9 + 1). The reference standards were spotted along with the samples. Meanwhile, the stock standard solutions of aflatoxins (10 µg/mL) were prepared in toluene + acetonitrile (9 + 1) all in accordance with procedures of AOAC (2006).

2.4. Purification and estimation of aflatoxins

The solvent system used (chloroform-acetone-propanol) has the following Rf-values for aflatoxin B_1 (0.66) and B_2 (0.59). The detected bands of aflatoxins (AFs) on the silica gel coated plates were marked according to the Rf-values for each aflatoxin.

For example, in the first plates: $B_1 = \frac{x}{15.80} = 0.66$ where x = distance travelled by B_1, 15.80 = distance travelled by solvent front, 0.66 = Rf-value for B_1. Therefore, $x = 15.80 \times 0.66$, $x = 10.43$. Also, $B_2 = \frac{x}{15.80} = 0.59$, where x = distance travelled by B_2, 15.80 = distance travelled by solvent front, 0.59 = Rf-value for B_2. Therefore: $x = 15.80 \times 0.59$; $x = 9.32$.

The required bands containing the aflatoxins were scrapped and placed into smaller centrifuge tubes and labelled accordingly. Four mL of methanol was added into each of the tubes. The mixture was shaken vigorously and thoroughly and centrifuged for 5 min at 3,000 rpm. The absorbences of the samples were taken at 350 nm and used to estimate concentrations of the aflatoxins by comparing with the absorbance of the known standard and read at 350 nm [26]. Also, three mL of methanol was added to each of the remainder of the reconstituted and re-dissolved residues with 2 mL benzene:acetonitrile mixture (9.8:0.2 mL) that were used to spot the plates. The resultant mixture was centrifuged for 5 min at 3,000 rpm, and the absorbences were read at 350 nm. The aflatoxin concentration was calculated according to the following equation: $AF\ (\frac{ug}{mL}) = \frac{A \times MW \times 1000}{\varepsilon}$, where A = absorbance at 350 nm, MW = molecular weight of the AFs (B1= 312, B2 = 314), ε = molar absorptivity at 350 nm. Each of the above tests was replicated three times.

2.5. Statistical analysis

All data collected were subjected to statistical analysis. The mean, standard error and least significant difference (LSD) at $p \leq 0.05$ (for comparing the aflatoxin levels of each sample) were determined using one-way analysis of variance (ANOVA). Two-way ANOVA was also carried out and the statistical significance ($p \leq 0.05$) between means of aflatoxin levels in samples and locations/markets was evaluated. Mean separation was done using Duncan multiple range test. All computations and statistical analysis were done using Genstat for Windows (Version 3.2).

3. Results

The aflatoxin B_1 content of the food samples from the markets showed that groundnut butter contained the highest aflatoxin B_1, while guinea corn cold pap produced the least and there were significant differences ($p \geq 0.05$) amongst samples. Samples from Orba market recorded the highest aflatoxin B_1 content, while that from Ogige market produced the least aflatoxin B_1 content with significant differences ($p \leq 0.05$) among markets (Table 1). The aflatoxin B_2 content of the various food samples from all the markets indicated that guinea corn pap contained the highest level of aflatoxin B_2, while maize pap contained the least with significant differences ($p \geq 0.05$) among samples. Also, samples from Orba market recorded the highest aflatoxin B_2 content with significant difference ($p \geq 0.05$) among markets (Table 2).

The data on the total aflatoxin content of the food samples from all the markets showed that peanut butter recorded the highest total aflatoxin content, while maize pap produced the least total aflatoxin content with significant differences ($p \leq 0.05$) among samples. Nkwo market produced the highest total aflatoxin content in peanut butter, while the least total aflatoxin content was recorded with the same market but in maize cold pap with significant differences ($p \leq 0.05$) among markets (Table 3).

Table 1. Aflatoxin B_1 contents (µg/kg) of maize pap, guinea corn pap and peanut butter from three markets in Enugu State, Nigeria

Location	Maize cold pap (*Akamu*)	Guinea corn cold pap (*Dawa*)	Peanut butter (*Ose oji*)
Nkwo	1.48 ± 0.20^{a1}	1.64 ± 0.08^{a2}	1.72 ± 0.48^{b3}
Ogige	1.48 ± 0.15^{a3}	1.25 ± 0.34^{a2}	0.94 ± 0.13^{a1}
Orba	2.73 ± 0.56^{b2}	1.56 ± 0.31^{a1}	4.29 ± 1.30^{c3}

Data is expressed as Mean ± SEM. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different numbers are significantly different ($p < 0.05$).

Table 2. Aflatoxin B_2 contents (µg/kg) of maize pap, guinea corn pap and peanut butter from three markets in Enugu State, Nigeria

Location	Maize pap (*Akamu*)	Guinea corn pap (*Dawa*)	Peanut butter (*Ose oji*)
Nkwo	0.78 ± 0.08^{a1}	1.89 ± 0.42^{a3}	1.10 ± 0.25^{a2}
Ogige	1.02 ± 0.30^{b1}	2.59 ± 1.86^{c3}	1.33 ± 0.41^{a2}
Orba	2.43 ± 0.15^{c3}	2.04 ± 0.20^{b1}	2.28 ± 0.26^{b2}

Data is expressed as Mean ± SEM. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$).

Table 3. Total aflatoxins contents (µg/kg) of maize pap, guinea corn pap and peanut butter from three markets in Enugu State, Nigeria

Location	Maize pap (*Akamu*)	Guinea corn pap (*Dawa*)	Peanut butter (*Ose oji*)
Nkwo	8.90 ± 0.9^{a1}	12.90 ± 2.30^{b2}	49.10 ± 0.73^{c3}
Ogige	22.20 ± 4.5^{b2}	26.40 ± 1.50^{c2}	40.00 ± 6.60^{c3}
Orba	14.4 ± 1.6^{b2}	9.70 ± 0.70^{a1}	22.80 ± 0.50^{a3}

Data is expressed as Mean ± SEM. Mean values in a column with different alphabets are significantly different ($p < 0.05$). Mean values in a row with different figures are significantly different ($p < 0.05$).

4. Discussion

The presence of aflatoxins (B_1 and B_2) in food samples collected from different local markets in Nsukka is in agreement with previous reports of contamination of food items from within and outside Nigeria [27, 28, 29, 30]. Some of the aflatoxin B_1 and B_2 detected in peanut butter exceeded the 2 µg/kg tolerable limit adopted by the European Union and other developed countries of the world, but were below the 5 µg/kg level set by FAO/WHO for

developing countries [31, 32, 33]. Despite these discrepancies in the levels of tolerable limits, aflatoxin B_1 a potent mutagen, has been classified as group 1 human carcinogen [34] and implicated in epidemics of acute hepatitis injury [35]. The consumption of peanut butter contaminated with aflatoxins, more especially aflatoxin B_1, beyond the recommended tolerable limits is unsafe. Aflatoxin B_1 is the strongest, as well as the most cancerogenous of all mycotoxins discovered so far. It is assumed to be associated with the occurrence of primary hepatocellular carcinoma in liver and there is synergistic interaction between hepatitis B virus and exposure to aflatoxin [36].

Generally, the total aflatoxin contents of most of the food samples in some of the locations were alarming as they exceeded the maximum tolerable limits for total aflatoxins in food (10 µg/kg) meant for human consumption set by the FAO/WHO for more than 75 countries around the world (including Nigeria) and far exceeds the 4 µg/kg standard of the European Union [31, 32, 33, 37, 38]. The differences in levels of aflatoxins observed among both food samples and localities could be influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods. It has been reported that hot, humid and wet climate encourage aflatoxin production in maize samples [6]. Agronomic factors reported to influence aflatoxin production include planting date, harvest time, pattern of cropping, variety, storage conditions, irrigation and plant stress [3]. Also, insect pests feeding on maize ears in the field and grains in storage might expose them to fungal spores, sporulation and aflatoxin contamination [39, 40]. Our results have revealed the great health risk consumers of maize/ guinea corn paps and peanut butter are exposed to in the study area. In another study carried out in Nigeria, blood and semen aflatoxins levels were alarmingly higher in infertile men than in fertile men, suggesting that aflatoxins could be a factor in infertility among humans [41].

It has been reported that even consistent low level consumption of mycotoxins could lead to serious human health problems including impaired growth and development, immune dysfunction and alterations in DNA metabolism [1]. The risk becomes even more worrisome since the semi liquids are mostly used as foods for babies and children whose immune systems and metabolism might not be as strong and efficient as those of the adults. The aflatoxin content of the peanut butter should be another source of concern because of the importance of peanut butter in ceremonies and social gatherings in the study area. The butter is eaten freely and in good quantities too during ceremonies and even at home because the people of the area relish the taste and aroma. Given this situation, it becomes necessary that more resources and enlightenment should be directed towards reducing the aflatoxin contents of foodstuffs to the barest minimum to ensure a healthy and productive populace. Certain practices such as sorting, dehulling, milling, winnowing and cleaning have been reported as effective measures that can reduce mycotoxins in cereals and grains [42]. Harvested grains should be dried immediately to the moisture content of 10-13% to avoid infection by toxigenic fungi during storage [43]. It is important that farmers and consumers of these foods alike should be educated on possible ways of storage that would discourage growth of storage fungi, especially *A. flavus* and *A. parasiticus* that have always been found associated with maize, guinea corn, and peanut contamination.

5. Conclusion

Unsafe levels of mainly the total aflatoxins were found in cereal cold paps and peanut butter sold in the study area.

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Conflict of Interest

There is no conflict of interest among the authors.

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