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Full Length Research Paper

Preliminary screening of aflatoxin level in maize (*Zea mays* L.) in some selected markets in Benue State, Nigeria

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Preliminary screening of aflatoxin B_1 levels in maize (*Zea mays* L.) sold in some markets of Benue State, Nigeria, was carried out using thin layer chromatography (TLC). Concentrated chloroform extracts of 10, 15 and 40 µl were used in diethylether - methanol - water solvent system in the ratio of 96:3:1. Moisture contents of the stored and oven-dried fresh maize collected from the markets were in the range of 7.64 to 13.40% which are below the maximum recommended level (13.50%) for stored maize. The result of TLC revealed that aflatoxin B_1 was not detected, as no spot was observed in these samples. This suggests that aflatoxin B_1 contents of both the oven-dried fresh and stored maize are either negligible or below the detection limit of 3.125 µg/kg for the TLC method employed. Hence, aflatoxin B_1 levels in the samples, if present, fall below the permissible limit (4 to 5 µg/kg) by National Agency for Food and Drug Administration and Control (NAFDAC) - Nigeria. Further review of this work will be carried out by the authors using a more sensitive technique like high performance liquid chromatography (HPLC) to detect the exact status of aflatoxins in maize from these markets as they are representative of maize belt in Benue State so as to compare them with other world standards.

Key words: Maize, aflatoxin B_1, *Aspergillus flavus,* thin layer chromatography (TLC), moisture content.

INTRODUCTION

Maize is one of the staple foods in this part of the country. More often than not, the maize is allowed to sun-dry on the farm before harvesting amidst unpredictable weather conditions (Nigerian Stored Products Research Institute, 1982). Also, the manner of storage is worrisome, as the maize are stocked in sacks and heaped in stores either at home or in market places. This is capable of creating congenial moisture content in the maize which can highly favour the growth of aflatoxin. Considering the implication of this, it will be appropriate to determine the levels of aflatoxin in maize produced in these localities of the country. Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus, the most notable ones being *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are toxic among the most carcinogenic substances known (Aflatoxin; From Wikipedia, the free encyclopedia (2012); Kerstin and Charity, 2011). After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M_1 (AFM_1). Of all the known mytoxins - toxic substances produced by fungi, aflatoxins in maize and other food items such as milk, peanuts, or cottonmeal, etc, generate the most concern when they occur at high levels. Out of the 14 different types of aflatoxins produced in nature, aflatoxin B_1 is considered the most toxic and is produced by both *A. flavus* and *A. parasiticus* (Aflatoxin; From Wikipedia, the free encyclopedia (2012); Oliveira et al., 2009). Aflatoxin G_1 and G_2 are produced exclusively by *A. parasiticus*. Aflatoxins M_1 and M_2 were originally discovered in the milk of cows that fed on moldy grain. These compounds are products of a conversion process

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in the animal's liver (Aflatoxin; From Wikipedia, the free encyclopedia (2012); Oliveira et al., 2009; Chemical Hazards Evaluation, 2001; Ehrlich and Lee, 1984).

Growth of aflatoxins is dependent upon weather conditions before or after harvest. Preharvest aflatoxin contamination of maize is associated with drought and temperatures during grain fill. When soil moisture is below normal and temperatures are high, the number of *Aspergillus* spores in the air increases. These spores infect crops through areas of damage caused by insects. Once infected, plant stress occurs and the production of aflatoxin is favoured. Postharvest aflatoxin contamination can develop when grain is improperly managed through the drying and storage processes under favourable conditions of humidity and temperature (Oliveira et al., 2009).

Marco et al. (2008) reported the use of an accelerated solvent extraction (ASE) system followed by on-line solid-phase extraction liquid chromatography (SPE-LC) for the analysis of aflatoxins in corn and almonds. ASE uses high temperatures during extraction to speed-up the extraction process, while incorporating high pressure to maintain the solvents in their liquid state. The on-line SPE-LC approach automates sample clean-up and aflatoxin analysis, increasing throughput while decreasing labour.

Mustard oil, used for cooking in northern India, was analyzed spectrophotometrically and 33 of 100 samples were found to contain aflatoxins at levels of 55 to 87 ppb. An early unpublished study of peanut oil in China showed that 48% of 1172 samples were positive for aflatoxin B_1, and a more recent long-term survey in Fujian province found 66% of 323 samples aflatoxin-positive, with 71 samples exceeding the Chinese tolerance level of 20 ppb aflatoxin B_1. Aflatoxin contamination of olive oil has received the most attention but the results have been contradictory. Analysis of 50 Greek olive oils demonstrated the presence of aflatoxin B_1 in 72% of the samples but the highest level detected was 0.05 ppb, well below the European Union (EU) regulation of 2 ppb (Noreen et al., 2011).

Earlier work had reported aflatoxin levels in Spanish olive oils, and in a selection of both Greek and Spanish oils, of 13 to 155 ppb and 5 to 10 ppb, respectively. More recently, total aflatoxin levels of 0.006 to 0.04 ppb were found in 46% of 28 Sicilian olive oil samples examined, and of 20 experimental and 15 commercial samples analyzed by LC-mass spectrometry (LC-MS) only 3 of the latter were contaminated, but below the method quantification limits for individual aflatoxins.

High performance liquid chromatography (HPLC) method with fluorescence detection developed for simultaneous analysis of aflatoxin B_1 and ochratoxin A showed that only 3 of 30 olive oil samples from southern Italy and Morocco contained aflatoxin B_1 at 0.5 to 2.4 ppb, whereas 80% contained ochratoxin A (Noreen et al., 2011). Cano-Sancho et al. (2010) determined AFM_1 in 72 composites of milk, 72 composites of cheese and 72 composites of yoghurt from Catalonia. AFM_1 content was analyzed using an Enzyme-linked immunosorbent assay commercial kit.

The current acceptance level of total aflatoxin in maize, set by the United States' Food and Drug Administration (USFDA) is 20 µg/kg (Tara, 2005; Reddy and Salleh, 2011; Paul and McNeill, 2010). The European Commission has set maximum levels for aflatoxin B_1 between 2.0 and 8.0 µg/kg and for the sum total of all 4 of these toxins between 4.0 and 15.0 µg/kg in crops such as nuts, groundnuts, grains and dried fruits (European Commission Regulation, 2006; Marco et al., 2008). However, the present study is limited to preliminary investigation.

The researchers will carry out a review of the study to establish the exact concentrations of aflatoxin B_1 using a more sensitive technique.

MATERIALS AND METHODS

The following materials and facilities were utilized during this studies: Viewing cabinet, 270 × 270 mm base equipped with 1.5 W long wave ultraviolet (UV) lamp 366 mm, thin layer chromatography (TLC) plate (20 × 20 cm glass plates coated with 0.25 mm silica gel), methanol, n-hexane, chloroform, anhydrous diethyl ether (100%), acetone, silica gel, sodium chloride, aflatoxin B_1 standard solution (0.5 µg/ml). All the reagents were of British Drug Houses (BDH) analytical grade.

Sample collection

The stored maize was sun-dried before packaging. However, moisture content was not determined prior to storage. Adequate pest control measures were taken in the stores. The stores were well constructed with controlled ventilation and protected from rain and humidity. The samples were collected 4 months after storage. Sampling of commodities was conducted according to the methods employed by Bainton et al. (1980), Makun et al. (2010) and Hurburgh (2005). A total of 256 samples comprising 128 stored maize and 128 fresh maize were collected from major markets; Gboko, Naka, Makurdi, Gbajimba, Ihugh, Zaki-biam, Katsina-Ala and Adikpo in Benue State.

The samples were collected between 1st week of October and 2nd week of November, 2011. Four (4) sampling stations were mapped out in each market and 4 samples were randomly collected from each sampling station. The level of aflatoxins B_1 was determined in total of 8 composites/category by pooling all the 16 samples from each market. Fresh maize samples were collected immediately they were brought from the farms.

The stored maize samples were collected from major warehouses (stores) from the sampling stations. About 0.2 kg of each sample was collected into labelled polythene bags and transported to the laboratory where they were stored at -4°C until analysis. Fresh samples were immediately conveyed to laboratory and oven-dried at 105°C intermittently until constant weight was observed (after 6 h) to avoid mould growth.

Sample pre-treatment

The samples were reduced to fine particles, using milling machine, and a sieved (<2 mm). Then, the moisture content of each of the sample was determined. The samples were kept in Erlenmeyer flasks prior to extraction.

Determination of moisture content

A crucible was weighed empty and its weight noted as W_1. Then, some quantity of the milled maize was put into the dish and the combined weight was measured as W_2. The weighed sample was immediately transferred to the oven and dried at 105°C intermittently until constant weight was obtained after which it was allowed to cool for 1 h and reweighed.

The final weight was noted as W_3 (Nigerian Stored Products Research Institute, 1982). The process was repeated for all the samples. The moisture contents were then calculated for all the samples, using the formula:

$$\text{Percentage moisture} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times 100$$

$$\text{Percentage moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Preparation of TLC plate

30.0 g of Silica gel was weighed into 250 ml glass-stoppered Erlenmeyer flask, and 100 ml of distilled water was added. This was shaken vigorously for 1 min and poured into the applicator set at 0.25 mm thick. This was used to coat five 20 × 20 cm glass plate. The plates were left undisturbed for about 10 min, and it gelled. The coated plates were transferred into the oven and kept for 2 h at 80°C to dry. The plates were stored in the desiccator's cabinet until just before use.

Sample extraction

25 g each of the milled maize samples was weighed separately into 250 ml glass-stoppered Erlenmeyer flasks. 100 ml methanol-water (5.7: 1) was added to each of the flasks, stoppered and protected with a foil. The flasks were shaken vigorously for 30 min. The mixture was filtered through a Whatman No. 1 filter paper and the residue was discarded.

Clean up

20.0 ml of the filtrate was transferred into a 125 ml - separating funnel, and 20.0 ml of 10% sodium chloride solution was added and mixed. 12.5 ml of n-hexane was added and the mixture was shaken for 1 min. Phases were allowed to separate. The lower phase (aqueous) was drained into a second 125 ml separating funnel, while the upper phase (organic) was discarded (Alexander et al., 1997).

Chloroform extraction

To the aqueous phase in the separating funnel, 12.5 ml of chloroform was added and shaken for 1 min. Phases were allowed to separate and the lower phase (organic) was allowed to pass through a bed rock of sodium sulphate into a 250 ml Erlenmeyer flask. The above steps were repeated for all samples.

Concentration of sample extract

After the chloroform extraction, the extracts were concentrated by evaporating to dryness on a steam bath. The concentrated extract was sealed with a foil and kept in a coolant.

Spotting of the TLC plate

The evaporated extracts were each dissolved in 250 µl of chloroform. Various volumes of the samples extracts 10, 15 and 40 µl and aflatoxin standards were spotted on the start line of the TLC plate for all the samples. The spotting was done using 1000 µl syringe.

Plates development and viewing

The plates were developed in a development tank presaturated with recommended development solvent (diethylether-methanol-water in the ratio 96:3:1). The development was carried out in the dark (cupboard) to avoid photo degradation of aflatoxin. The spotted plate was placed vertically in the development tank and covered properly. It took 35 to 45 min for the solvent to reach the stop line (10 cm) from the base line. The plate was then removed and allowed to dry. This process was repeated for all the samples and the developed plates were viewed under a long wavelength UV lamp (366 nm).

RESULTS AND DISCUSSION

The results for moisture contents and levels of aflatoxin B_1 of maize samples obtained from selected markets in Benue State are shown in the Table 1.

The results of the moisture contents determination of the maize (Table 1) revealed that the moisture levels of the fresh oven-dried maize varied between 7.64 to 10.16%, while those of the stored maize varied between 9.96 to 13.41%. Stored maize samples collected from Ihugh and Adikpo markets recorded the lowest and highest moisture levels, respectively. However, these values were below the recommended moisture limit of 13.5% for stored maize (Eaton and Cropman, 1994; Nigerian Stored Products Research Institute, 1982). The low moisture contents of the maize observed in this study may have significant retardation effect on the growth of the fungus (*A. flavus*) responsible for the production of aflatoxin (Aflatoxin; From Wikipedia, the free encyclopedia (2012); Oliveira et al., 2009).

The TLC determination of the levels of aflatoxins in both the fresh and stored maize in Tables 1 revealed that aflatoxin was not detected for the volumes of 10, 15 and 40 µl of extracts spotted. This suggests that maize from areas sampled may be free from aflatoxin B_1 contamination or if present, the contamination may be below the detection limit of 3.125 µg/kg of the TLC method employed (Nigerian Stored Products Research Institute, 1982; Alexander et al., 1997). Furthermore, the low moisture levels of the maize may not be favourable for the growth of mould that enhances aflatoxin B_1 contamination.

Conclusion

The results of the preliminary investigation of aflatoxin levels in some fresh oven-dried and stored maize obtained from most prominent maize producing areas of

Table 1. Moisture contents and levels of aflatoxin B_1 of maize samples obtained from selected markets in Benue State.

Maize sample	Moisture content (%)	Aflatoxin B_1 (µg/kg)
Stored samples of Gboko	12.83	ND
Fresh samples of Gboko	10.16	ND
Stored samples of Naka	12.40	ND
Fresh samples of Naka	7.64	ND
Stored samples of Makurdi	11.70	ND
Fresh samples of Makurdi	9.70	ND
Stored samples of Gbajimba	11.90	ND
Fresh samples of Gbajimba	8.56	ND
Stored samples of Ihugh	9.96	ND
Fresh samples of Ihugh	8.53	ND
Stored samples of Zaki-biam	13.40	ND
Fresh samples of Zaki-biam	9.68	ND
Stored samples of Katsina-Ala	13.21	ND
Fresh samples of Katsina-Ala	9.63	ND
Stored samples of Adikpo	13.41	ND
Fresh samples of Adikpo	9.94	ND

ND, Not detectable.

Benue State, Nigeria revealed that the stored maize had lower residual moisture than the recommended 13.5% maximum moisture for maize storage. The low level of moisture contents of maize samples may significantly discourage mould infection and aflatoxin development in the maize. No aflatoxin B_1 was detected using the TLC method employed and this showed that even if aflatoxin B_1 were present, it may have been below the detection limit (3.125 µg/kg) of this technique.

RECOMMENDATION

Further study on this subject will be required using a more sensitive equipment such as HPLC with a detection limit of 0.10 µg/kg (Oliveira et al., 2009).

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