



Full Length Research Paper

Total aflatoxin level and fungi contamination of maize and maize products

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ABSTRACT

Samples of maize and maize products purchased from selected markets in parts of Kaduna State, Nigeria were analyzed for total aflatoxin and fungi contamination using Enzyme Linked Immunosorbent Assay (ELISA) and mycological procedures. The mean aflatoxin concentration of the maize and maize products obtained from this study shows that one hundred and fifty two (82.70%) of the maize products had aflatoxin contamination above the tolerance level of 20ppb stipulated by World Health Organization). The old maize had a mean aflatoxin level of 177ppb while the new maize had a mean aflatoxin level of 102ppb. The mean aflatoxin level in maize flour was 174ppb while the maize bran had a mean aflatoxin level of 213ppb. Significant differences exist between the mean aflatoxin contaminations of old maize, fresh maize, maize flour and maize bran. Five fungal genera namely: *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Botrytis* were isolated. *Aspergillus* was the most predominant genera isolated (62%). Among the *Aspergillus*, three species namely *A. niger*, *A. tamari*, and *A. flavus* were identified. The high level of aflatoxin in maize and maize products highlights the health problem associated with the consumption of these products with the toxin that could lead to adverse health effects such as liver, cervical, breast or oesophageal cancer.

Keywords: Maize, Aflatoxin, ELISA, Contamination, Fungi

INTRODUCTION

A large quantity of food are wasted every year, because they are invaded by toxic fungi and get contaminated by fungal metabolic products. Such spoilage is prominent in hotter countries, where problems of food shortages already exist. One reliable estimate as reported by CAST, (2003) indicated that mycotoxins affect a quarter of the world's food crops, including many basic foodstuffs such as animal feed, crops like maize, rice and wheat. Mycotoxins are natural secondary metabolites produced by fungi on agricultural commodities in field and during storage, under a wide range of climatic conditions and are of significance in food safety (Mady, 2001). The food borne mycotoxins that are likely to be of great significance to human health in tropical developing countries are the fumonisins and aflatoxins (COT, 1997). Among these mycotoxins, aflatoxin has gained considerable attention because it is a more toxic and potent carcinogen even in small quantities (FAO, 1979), and it is also the mycotoxin of public health importance within the West African region (WHO, 1999).

Aflatoxins are toxic metabolites produced by a variety of moulds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Crops may be contaminated with one or more of the four following sub-types of aflatoxin: B₁, B₂, G₁ and G₂. Aflatoxin B₁ is the most toxic and frequently detected form. The other types present a significant danger at a high level concentration. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of feeds that are contaminated with aflatoxin producing fungal strains during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, decreased milk and egg production. Most controlling government agencies worldwide have

regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of the presence of aflatoxin in commodities is of paramount importance.

The Standards Organization of Nigeria (SON) sets standards on many food commodities, taking into account global standards as well as national production and target export markets. While it is generally recognized globally that there is no safe level of aflatoxin exposure, SON has set the maximum acceptable limit for maize grain at 4 ppb for total aflatoxins and 2 ppb for aflatoxin B1. The FDA action level of aflatoxin in agricultural products is based on the opinion made in the late 1960s that an unavoidable potential human carcinogen in important food stuffs should be controlled at the lowest practical level. With regards to products for human consumption, the current action level is 20ppb total aflatoxin in a finished product while that of milk and milk products is 0.5ppb aflatoxin M₁. When these levels are exceeded, commodities are restricted to lower value uses usually animal feeds, oil products, and alcohol production (Park and Potland, 1986). The objective of this study is to determine total aflatoxin level and fungal contamination of maize and maize products. Findings of this studies will serve the purpose of alerting consumers on the dangers of consuming maize and maize products on sale in some selected markets within Kaduna State, Nigeria.

MATERIALS AND METHODS

Collection of maize and maize Product Samples

Maize and maize products for this study were purchased randomly from selected wholesale markets (grain markets) within Kaduna State. The samples were collected in into a low density sterile polythene bags with proper identification and transported to the laboratory for analysis.

Enumeration and characterization of moulds in maize and maize products

Twenty five (25g) of each homogenized sample using stomacher was taken aseptically and weighed into a Erlenmeyer flask containing 225ml of 0.1% sterile peptone water. This was allowed to remain for 2 - 3 minutes with occasional stirring using sterile glass rod. Ten fold serial dilutions were prepared by transferring 1ml aliquot of the supernatant into 9ml of sterile peptone water as diluents. Further serial dilution was carried out and thereafter, 0.1ml of appropriate dilutions were plated aseptically using Spread plate method on Potato Dextrose Agar [Biotech] supplemented with chloramphenicol. Inoculated plates were incubated at 28 ±2°C for 5-7 days. Number of colonies after incubation

were identified, counted and expressed as colony forming unit per g (cfu/g). The fungi that developed were purified by repeated subculturing. Pure cultures of fungi were examined macroscopically, microscopically and isolates were confirmed by comparing with the existing cultures already identified by the International Mycological Institute through the use of mycological atlas. Isolates were stored at 4°C in refrigerator until required. Presumptively identified *Aspergillus* species were sent to Commonwealth Agriculture Bureau International (CABI) Institute, London to further confirm the species.

Determination of total aflatoxin levels in maize and maize products

Total aflatoxin levels in maize and maize products were determine using standard kit (Aflatoxin plate kit (PN53012B)

Preparation of Solution for extraction

Twenty (20ml) of distilled water was carefully measured and transferred into 250ml clean glass container with a tight fitting lid. Eighty (80ml) of methanol was measured and added to 100 ml of extracting solution being prepared. It was then covered and shaken to mix completely and was stored tightly sealed to minimize evaporation.

Sample extraction and Extract dilution

Fifty grammes of ground maize sample was mixed with 5.0g NaCl and 100ml of 80% methanol : water (80:20) and blended in a blender jar at a high speed for 1 minute with the blender covered and 10 ml was filtered through a glass fiber filter.

Five milliliters (5 ml) of the filtered extract was collected in a clean vessel and diluted with 20ml of water and mixed thoroughly and filtered through a glass fiber filter

ELISA test for Total aflatoxin level

The total aflatoxin level in maize grains and maize products (maize flour and maize bran) were detected and quantified using Enzyme Linked Immunosorbent Assay (ELISA) kits (Helica Biosystem Inc.USA).The kit was used according to the manufacturer's instruction.

RESULT

The identification of the fungal isolates based on colonial appearance and microscopy is as shown in

Table 1. Cultural and morphological characteristics of the maize and maize product mould isolates

| Isolates | Colour on PDA | Growth rate | Vesicle | Conidia head | Conidiophores | Fungal isolates |
|----------|-------------------------|-------------|--------------|--------------|--|-------------------------|
| 1 | black | rapid | globose | Biseriate | Long, smooth conidiophore | <i>A niger</i> |
| 2 | Brown | rapid | Semi globose | Uniseriate | Long smooth conidiophore | <i>A tamari</i> |
| 3 | Green | Rapid | Semi globose | Biseriate | Long and smooth | <i>A flavus</i> |
| 4. | Leafy green | Rapid | globose | Biseriate | Long smooth and branched conidiophore | <i>Penicillium</i> spp. |
| 5 | Spongy wooly dark brown | Rapid | globose | Biseriate | Simple, long and branched conidiophore | <i>Rhizopus</i> spp |
| 6 | Pinkish brown | Rapid | globose | Biseriate | Long, smooth and branched conidiophore | <i>Fusarium</i> spp |
| 7 | Gray | Rapid | Semi globose | Biseriate | Long, smooth conidiophore | <i>Botrytis</i> spp. |

Source of guide: Mycological Atlas (Klich, 2002).

Table 2. Frequency and distribution of moulds isolated from maize and maize products

| Frequency and distribution of isolated mould (%) n = 157 | | | | | | |
|--|---------------------------|------------------------|---------------------------|------------------------|------------------------|-----------|
| Sample | <i>Aspergillus</i> Spp | <i>Fusarium</i> Spp | <i>Penicillium</i> Spp | <i>Rhizopus</i> Spp | <i>Botrytis</i> Spp | Frequency |
| Old Maize | 21 | 1 | 3 | 6 | 2 | 33 |
| New Maize | 24 | 2 | 3 | 11 | 4 | 44 |
| Maize Flour | 20 | ND | 1 | 4 | 1 | 26 |
| Maize bran | 32 | 2 | 2 | 14 | 4 | 54 |
| Total | 97 | 5 | 9 | 35 | 11 | 157 |
| Percentage (%) | 62 | 3 | 6 | 22 | 7 | 100 |

ND – Not Detected

Table 1. Colonial morphology was characterized by radial fissures with smooth and entire edges. The microscopic morphology revealed long/smooth conidiophores, globose vesicles and conidia head being biseriate or uniseriate.

The frequency and distribution of mould isolated from maize and maize products is shown in Table 2. Five mould genera were identified, *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus* and *Botrytis*. *Aspergillus* accounts for 62% of the mould types encountered in the study while other mould types are *Fusarium*, (3%) *Penicillium* (6%), *Rhizopus* (22%) and *Botrytis* (7%).

The mean aflatoxin concentration of the maize and maize products obtained for this study is shown in Figure 1. One hundred and fifty two (82.70%) of the maize products had aflatoxin contamination above the tolerance level of 20ppb fixed by World Health Organization (WHO, 1979). The old maize had a mean aflatoxin level of 177ppb while the new maize had a mean aflatoxin level of 102ppb. The mean aflatoxin level in maize flour was 174ppb while the maize bran had a mean aflatoxin level of 213ppb.

DISCUSSION

This study investigates total aflatoxin level and contamination of maize and maize product.

Maize and maize products have been documented by several authors as an excellent substrate for mould growth and aflatoxin contamination in some West African countries including Nigeria.

The major genera of fungi found to contaminate maize and maize products in this study comprise of field and storage. The isolation and identification of five fungal species which are of field and storage importance will go a long way in minimizing fungal infestation of grains. (Almeida *et al.*, 2000, Bankole and Kpodo, 2005). The occurrence of *Aspergillus* sp, *Penicillium* and *Fusarium* spp in maize was earlier reported in South Africa (Marasaset *et al.*, 1981), Cameroon (Ngoko, *et al.*, 2001), and in Nigeria (Onyeke and Nelson 1992).

Muthoni *et al.* (2009) reported *Aspergillus* and *Penicillium* spp. as common storage fungi. These fungi genera are well known as maize contaminants worldwide (Miller, 1995; Joseph and Kolapo, 2008). The distribution

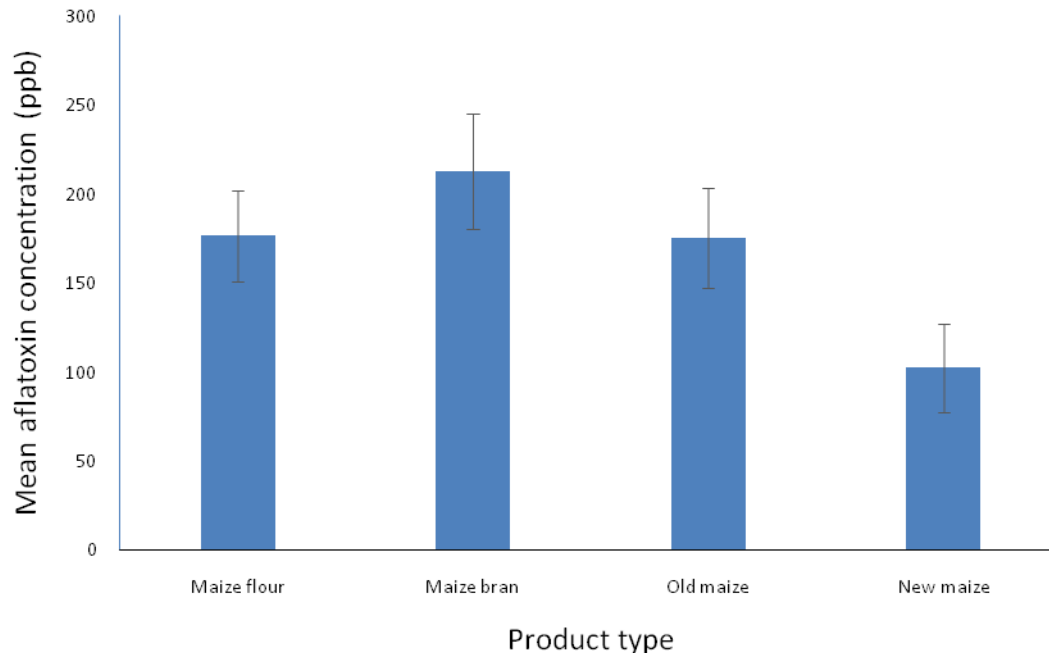


Figure 1. Mean aflatoxin concentration in part per billion (ppb) of maize and maize products

of fungal isolates, in this study showed that *Aspergillus* species was the most dominant of the fungi genera isolated. Maize infestation by this mould is common and widespread (Amadi and Oso 1996; Almeida *et al.*, 2000, Kpodo and Bankole ,2005).This may be due to wide range growth requirements of this species and also its ability to grow on a minimal medium. This finding is in agreement with the earlier report of Bankole *et al.*,(2003).

Maize is one of the high risk crops for aflatoxin contamination worldwide (Setamon *et al.*, 1987). Most countries of the world regulate total aflatoxin in all food at 20ppb (FAO, 1997).

The result obtained shows high level of aflatoxin contamination in all the samples above the acceptable aflatoxin level of 4ppm for maize set by Standard Organization of Nigeria (SON) since (82.7%) of the products have demonstrated aflatoxin levels above 20 ppb, the acceptable level established by (FDA, 1994) and (FAO, 1997). Only 17.3% of the samples had levels below 20ppb which is considered as safe level for human or animal consumption. High level is said to be unacceptable and possible legal action to eliminate such products from market may be taken by the government (FAO, 2000). Among staple cereals in the Nigerian diet, maize was reported to have the highest level of aflatoxin contamination (Bandyopadhyay *et al.*, 2007). The high level of aflatoxin contamination recorded in this study may be attributed to several factors, such as temperature, longer storage time and other environmental factors. The high level of aflatoxin contamination of maize and maize products has been

widely reported by many researchers (FAO, 1979; Samson, *et al.*; 1981.,Bankole and Kpodo, 2005 and Groopman, 2008).

The aflatoxin producing organism *Aspergillus flavus* which occurred in 34% of the samples in the present study is in agreement with earlier observation that mycotoxins are highly stable and could be detected long after the producing fungi have died out or replaced by other fungi due to ecological succession.

Milling process as to obtain products for human consumption has an important effect in mycotoxin reduction. Corn meal and corn flour which are the principal products of this process had lower content of aflatoxin than unprocessed grain. The present study is in agreement with this, but the by-product such as maize bran was found to be more contaminated. This is not surprising, as aflatoxin is known to concentrate in the maize bran (Pietri *et al.*, 2009).The lower aflatoxin contents in maize flour as observed in this study are in line with the findings of other researchers (Furlong*et al.*, 1999). The traditional method that involves the removal of pericarp from maize to make maize flour could simply mean the removal of fungi associated with mycotoxin production (Fandohan *et al.*, 2005).The overall high level of aflatoxin contamination reported in this study is in agreement with those reported from previous studies in Benin Republic and Nigeria (Setamou *et al.*, 1987; Hell *et al.*, 2003). Similarly, such high level of aflatoxin in maize has been recorded in Kenya where an outbreak of acute aflatoxicosis was linked to the severe contamination level (Lewis *et al.*, 2005).Maize samples collected from soil

and warehouses were all reported to be contaminated with aflatoxin in the range of 20-355µg/kg (Kpodo ,1996). High levels of aflatoxin contamination have also been influenced by agronomic and biotic factors. Biological control of aflatoxin contamination through competitive exclusion of aflatoxin producers by toxigenic strains of *Aspergillus flavus* might provide the greatest reduction in contamination, where fungal communities have the highest average aflatoxin producing potentials (Cotty, 2006).

CONCLUSION

The result of this study revealed that most of the product (maize and maize product) obtained for the study have high aflatoxin levels than the 20ppb recommended FDA level. Household maize represents a significant source of exposure to aflatoxin. Sensitizing the commodity trader and the populace on the danger with the consumption of high level of aflatoxin in these products is important.

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