AFLATOXIN CONTAMINATION OF MAIZE, PEANUTS AND THEIR PRODUCTS IN ACCRA, GHANA

By

KPODO, K., ALLOTEY, E., ANTONIO, S. AND ANYEBUNO, G.

FOOD RESEARCH INSTITUTE (C.S.I.R.)

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SUMMARY

Samples of groundnuts, maize and their products obtained over a two-year period of time (2003-2004) were analysed for aflatoxins B_1, B_2, G_1, and G_2 by High Performance Liquid Chromatography. Analyses of a total of 91 groundnut samples indicated 37 samples containing more than 4 µg/kg (the EU maximum permissible limit) for aflatoxins with 30 samples exceeding the Ghana Standards Board maximum permissible limit of 15 µg/kg. Groundnut products analysed consisted of 34 groundnut snack products and 30 groundnut paste or butter samples. Aflatoxins were not detected in 14 of the 34 snack products and 5 of the 30 paste or butter samples. However 20 of the 30 paste samples contained over 15 µg/kg total aflatoxins whilst only three of the 34 snack samples contained more than 15 µg/kg. The highest total aflatoxin levels recorded for groundnut kernels and groundnut products were 24,873 µg/kg and 1,260 µg/kg respectively.

Analyses of nineteen maize kernel samples and five maize products confirmed earlier studies about high aflatoxin contamination levels. Thirteen of the nineteen maize samples had total aflatoxin levels exceeding the Ghana Standards Board maximum permissible limit of 15 µg/kg. The highest total aflatoxin level recorded for maize and maize products in this study was 1,156 µg/kg. It is evident from this study that aflatoxin contamination in groundnuts, maize and their products is common and pose a threat to both international trade and the health of consumers. There is therefore an urgent need for further research to monitor and manage the aflatoxin problem in the country.

INTRODUCTION

The aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus*, fungi distributed world-wide in the air, soil, and water. They can infect crops in warm, humid climates, with subsequent production of aflatoxins during growth, harvest, storage, transport, and processing by the consumer prior to use. *A. flavus* is adapted to an aerial environment and commonly infects maize, cottonseed and tree-nuts with subsequent production of aflatoxins B_1 and B_2 only, whilst *A. parasiticus* is adapted to a soil environment and commonly infects groundnuts, with production of aflatoxins B_1, B_2, G_1, and G_2.

Ghana is a tropical country and atmospheric conditions of temperature and relative humidity are favourable for the growth of these fungi and the production of aflatoxins in most agricultural commodities. The ingestion of food containing aflatoxins may have adverse health effects in both humans and animals. Aflatoxins are known to be hepatotoxic, carcinogenic and teratogenic.

In Ghana, maize is considered to be the most important staple food-grain (Prempeh, 1971) and is currently the most widely cultivated cereal crop in the country with a total annual production of 1.014 million metric tonnes (FAO, 2001). As a dietary staple, it contributes the major part (90 to 95%) of the total calories in the diet of Ghanaians especially in the coastal areas of Ghana (National Food and Nutrition Board, 1962). Maize is consumed in various forms ranging from immature grain eaten off the cob after cooking to products processed from matured kernels by indigenous technologies such as fermentation for products such as "Kenkey", "Banku", "Agidi" and "Koko" among others (Nyako, 1977). Groundnut is the most important oilseed in the developing world, and is a valuable source of protein for human and animal nutrition. In Ghana, groundnuts are cultivated mostly by subsistence peasant farmers in all the agro-ecological zones with the bulk coming from the Guinea and Sudan Savannah zones in the northern half of the country. In Ghana groundnut is an important source of food protein and oil. Most of the groundnut produced is processed by local women into peanut butter (paste) which is used for soups, vegetable oil, and to some extent used as spreads. Groundnuts are also roasted, boiled or homogenised in combination with cereals and condiments and eaten as a snack or delicacy. Currently Ghanaian mothers are being urged to incorporate peanuts and other grain legumes (cowpeas) into traditional weaning formulations for their infants to improve the protein quantity and quality to curb Protein-Energy malnutrition. Components of traditional and improved weaning foods in Ghana are therefore maize or sorghum with peanuts and cowpeas.

In view of the health effects of aflatoxins on humans, the International Agency for Research into Cancer (IARC) of the World Health Organisation has classified aflatoxins as Group 1 carcinogens. Apart from the health effects, aflatoxins are barriers to trade and the World Bank estimated annual trade losses of $670 million for nine African countries because the European Union (EU) recently reduced regulatory limits for aflatoxins to 4 ppb. The Codex Alimentarius Commission has also set a level of 20 ppb. In addition, several countries have established maximum permissible levels for various agricultural commodities. In Ghana, the Ghana Standards Board has set a limit of 15 ppb for total aflatoxins.

In line with the Government's export drive, a lot of emphasis has been placed on non-traditional export crops and currently Ghana exports groundnuts and groundnut products to several European Union and neighbouring countries. This paper presents the results of studies conducted to ascertain the aflatoxin contents of maize and groundnut kernels and their products destined for local consumption and for export.

MATERIALS AND METHODS

Maize and groundnut samples

Samples of maize, groundnuts and their products were obtained from local industrialists, and exporters over a two year period of time (2003 - 2004). A total of 91 groundnut kernel samples and 64 groundnut products were analysed for aflatoxins. Groundnut products consisted of roasted and coated groundnut snacks and groundnut paste/butter. Nineteen maize kernel samples and five maize products consisting of *Kenkey*, fermented maize flour and roasted maize flour were also analysed. Samples were milled, thoroughly mixed, and portions extracted and analysed for aflatoxins B_1, B_2, G_1, and G_2.

Extraction and clean-up procedure of samples

The aflatoxin extraction procedure was based on that of Pons (1979). Finely ground maize kernels/maize products were extracted with methanol followed by precipitation of colour pigments using zinc acetate then extraction into dichloromethane and further clean-up by column chromatography using cellulose and silica gel. Aflatoxins were eluted with dichloromethane (80:20 v/v) which was evaporated off and the residue quantitatively transferred into 10 ml of High Performance Liquid Chromatography (HPLC)-grade dichloromethane. Five millilitres were evaporated to dryness under a stream of nitrogen and the final residue dissolved in 0.1 – 1.0 ml HPLC mobile phase and used for HPLC analysis. The same extraction procedure was used for peanut kernels and products, except that hexane was added to the extraction mixture due to the high fat content of peanuts.

High Performance Liquid Chromatography (HPLC) analyses

The Instrument system used was from Waters Associates (Milford, MA, USA) and included a Model 515 Solvent Delivery System fitted with a Rheodyne Model 7725i injector with a 20 µl fixed volume loop, a temperature control system consisting of a temperature control module and two column heaters, a Model 474 Scanning Fluorescence Detector and a Model 746 Data Module.

Identification and quantification of aflatoxins was by reversed-phase liquid chromatography with post-column derivatisation. Separation of aflatoxins was carried out on a Spherisorb S5 ODS-1 column of dimension 25 x 4.6 mm packed with 5 µm particles (Phase Separations Inc., Norwalk, USA) maintained at 35°C. HPLC mobile phase consisting of methanol-acetonitrile-water (10:30:60, v/v/v) was pumped at a flow rate 1.2 ml/min. Post-column derivatisation of aflatoxins B_1 and G_1 was achieved using saturated iodine solution (Shepherd and Gilbert, 1984) pumped at a flow rate of 0.4 ml/min using an Eldex precision metering pump (Eldex Laboratories Inc., San Carlos, USA). The derivatisation tube consisted of a stainless steel tubing (5 m x 0.3 mm) maintained at 75°C.

The excitation and emission wavelengths used were 360 and 440nm respectively. The aflatoxins were identified by comparison of their retention times with those observed for the individual aflatoxin standards. Quantification was achieved by comparison of the peak areas of the extracts with those of the aflatoxin standards. To compensate for any day-to-day variation, standards were routinely run at the beginning, in-between and after samples had been run. The concentration of aflatoxins present in the samples was calculated using the equation:

$$\text{Concentration aflatoxin } (\mu g/kg) = A_p/A_s \times C_s \times V \times F \times 1000/m$$

Where A_p = peak area of extract

A_s = peak area of standard

C_s = concentration of aflatoxin in standard solution in µg/ml

V = volume of mobile phase used to dissolve extract (ml)

F = dilution factor

M = weight of milled sample extracted (25g)

Chemicals, reagents and standards

Aflatoxin standards were obtained from Sigma Chemical Co. Ltd. (St Louis, MO, USA). Standard stock and HPLC working solutions were prepared by evaporating and dissolving in the appropriate mobile phase consisting of methanol-acetonitrile-water (10:30:60, v/v/v). Reagents for HPLC separations were of the HPLC grade. All other chemicals and reagents used were of the analytical grade. Distilled and deionised water were used throughout and the mobile phase solution was filtered through a 0.45 µm Millipore HV disc filter and degassed prior to use through a Millipore filtration unit (Millipore Corp., Bedford, MA, USA).

RESULTS AND DISCUSSION

Table 1 shows the distribution of groundnut kernels and groundnut products at different levels of aflatoxins.

Table 1: Distribution of groundnut kernels and groundnut product samples at different levels of aflatoxins

Total Aflatoxin Range (µg/kg)	No. of groundnut kernel samples	No. of groundnut product samples
None Detected, (ND)[1]	26	19
≤ 4	28	17
5 - 15	7	5
16 - 25	2	4
26 - 100	7	7
101 - 500	14	9
> 501	7	3
TOTAL NO. OF SAMPLES	**91**	**64**

[1]ND, None detected. Detection limits, B_1 and B_2 = 0.04 µg/kg, G_1 and G_2 = 0.06 µg/kg

A total of 91 groundnut kernels were analysed during the two year period. Of this number 65 (71%) contained aflatoxins at varying levels. Fourteen samples contained between 101 and 500 µg/kg whilst seven samples contained over 501 µg/kg total aflatoxins. The highest total aflatoxin level recorded in this study was 24,873 µg/kg. It is known that the critical factors for the production of aflatoxins are substrate moisture content and relative humidity. The limiting relative humidity for aflatoxin production by *Aspergillus flavus* in groundnuts is 85% (Diener and Davis, 1967). In Ghana studies conducted by Kpodo and Gyato, (1997) have shown that after harvesting, peanuts together with the haulms are heaped and manually threshed after which the peanuts are sun dried. Drying could take as long as sixteen days, a situation creating conditions for the growth of *A. flavus* and *A. parasiticus* and production of aflatoxins.

It is also known that physical removal of mouldy, damaged, immature and shrivelled groundnut kernels reduces aflatoxin levels (Mintah and Hunter, 1979). Groundnut farmers in the major growing areas in Ghana tend to sort only those groundnuts reserved for use as planting material or seed (Kpodo and Gyato, 1997). This practice may account for the rather high contamination levels. In the current study, 37 samples (40%) did not meet the current European Union maximum permissible total aflatoxin limit of 4µg/kg. This revelation confirms the occasional rejection of groundnut kernel exports from Ghana by major trading partners in the EU countries through the Rapid Alert System for Food and Feed (RASFF). Thirty groundnut kernel samples (33%) contained over 15µg/kg, the maximum limit set by the Ghana Standards Board for aflatoxins.

For the groundnut products, 45 (70%) of the 64 samples analysed contained aflatoxins (Table 1). Twenty-eight samples (44%) contained total aflatoxin levels above the EU permitted limit whilst 23 samples (36%) did not meet the Ghana Standards Board limit of 15 µg/kg. This implies that processing of kernels into various products does not necessarily remove all aflatoxins if any at all.

Table 2 shows the distribution of groundnut products at different levels of aflatoxins.

Table 2: Distribution of groundnut product samples at different levels of aflatoxins

Total Aflatoxin Range (µg/kg)	No. of roasted and coated groundnut samples	No. of groundnut paste / butter samples
None Detected, (ND)[1]	14	5
≤ 4	13	4
5 - 15	4	1
16 - 25	0	4
26 - 100	2	5
101 - 500	1	8
> 501	0	3
TOTAL NO. OF SAMPLES	**34**	**30**

[1]ND, None detected. Detection limits, B_1 and B_2 = 0.04 µg/kg, G_1 and G_2 = 0.06 µg/kg

A total of 34 groundnut snack (roasted and coated) samples were analysed for aflatoxins of which 20 (59%) contained aflatoxins. Only seven samples (21%) however contained more than EU limit of 4 µg/kg total aflatoxins. Three samples did not meet the Ghana Standards Board maximum limit of 15 µg/kg. In contrast however, for groundnut paste/butter, of a total of 30 samples analysed, 21 (70%) contained over 4 µg/kg, whilst 20 (67%) contained more than 15 µg/kg and therefore did not meet the Ghana Standard. Three of the samples contained over 500 µg/kg with the highest value being 1,260 µg/kg. As indicated earlier, groundnut paste which is widely consumed throughout the country in the form of soups, stews, vegetable oil and also as spread is produced at the artisanal level by local women. Such high levels of aflatoxins are unacceptable and strategies have to be put in place to prevent contamination or to lower levels.

Table 3 shows the distribution of maize kernels and maize products at different aflatoxin levels.

Table 3: Distribution of maize kernels and maize product samples at different levels of aflatoxins

Total Aflatoxin Range (µg/kg)	No. of maize kernel samples	No. of maize product samples
None Detected, (ND)[1]	0	0
≤ 4	4	0
5 – 15	2	4
16 – 25	0	0
26 – 100	6	1
101 – 500	1	0
> 501	6	0
TOTAL NO. OF SAMPLES	**19**	**5**

[1]ND, None detected. Detection limits, B_1 and B_2 = 0.04 µg/kg, G_1 and G_2 = 0.06 µg/kg

Nineteen maize kernel samples were analysed of which 13 (68%) did not meet the Ghana Standard of 15 µg/kg. Six samples contained over 501 µg/kg total aflatoxins with the highest level being 1,156 µg/kg. Five maize products were analysed with one sample containing over 15 µg/kg. Total aflatoxin content of this sample was 80 µg/kg. This study confirms earlier findings about the presence of aflatoxins in maize and fermented maize products in Ghana (Kpodo and Halm, 1990; Kpodo *et al.*, 1996).

CONCLUSION

Aflatoxins have been detected in maize and groundnut kernels and their products at unacceptable levels. Efforts have to be directed at the education of farmers and processors about the existence of aflatoxins in agricultural commodities and their health effects on humans and animals. In addition, training sessions on simple but practical ways of avoiding and reducing contamination need to be introduced if the Government's agricultural export drive is to be achieved.

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