Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **1(8),** 1-5, Nov. **(2011)** *Res.J.Chem.Sci.*

Enzyme - Linked Immunosorbent Assay (Elisa) of Aflatoxin B1 in Groundnut and Cereal Grains in Lagos, Nigeria

Ayejuyo O.O.[1], Olowu R. A.[2], Agbaje T.O.[2], Atamenwan M.[2] and Osundiya M.O.[2]
[1]Chemistry Department, University of Lagos, Akoka, NIGERIA
[2]Chemistry Department, Lagos State University Ojo, Lagos, NIGERIA

Available online at: www.isca.in
(Received 23rd May 2011, revised 23rd September 2011, accepted 27th September 2011)

Abstract

Enzyme linked immunosorbent assay (ELISA) technique was used to assess the levels of aflatoxin B1 in groundnut and cereal grains commonly consumed by wide majority of Nigeria populace. Homogenous samples purchased from different markets in Lagos were extracted with 70% (v/v) methanol. The extracted samples and HRD-conjugated aflatoxin B1 were mixed and added to the antibody coated microwell. On removal of non-specific reactants, TMB substrate was added and the microwells measured optically by microplate reader at 450nm. The results showed that aflatoxin contents of groundnut ranged from 6.25 ng/g to 7.80 ng/g. The levels of this substance were 4.18 ng/g and 28.50 ng/g in millet samples. The maize samples contained between 2.51 ng/g and 3.94 ng/g aflatoxin as against the 5.20 ng/g found in sorghum. About fifty one percent aflatoxin incidence was found in the 99 samples investigated. With a safe limit of 20 ng/g set by food and drug administration agency, only the millet samples would prove toxic to the consumers of these products.

Keywords: Aflatoxin B1, food products, enzyme linked immunosorbent assays (ELISA)

Introduction

Aflatoxins are toxic compounds produced as secondary metabolites by the fungi Aspergillus flavus and Aspergillus parasiticus growing on a variety of food products. Among the 18 different types of aflatoxins identified, the major ones are aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2 [1-5]. The international agency for research on cancer (IARC) classified these four aflatoxins as group 1 carcinogens[6]

Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive and can be found in grains, nuts, cottonseed and other commodities associated with human food or animal feeds[1,3,7]. Crops may be contaminated by one or more of the four sub-types of aflatoxin: B1, B2, G1 and G2. Aflatoxin B1 is the most toxic and the most frequently detected form. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Reye's syndrome and chronic hepatitis[8]. Animals are exposed to aflatoxins by consumption of feeds that are contaminated by aflatoxin-producing fungal strains. Aflatoxins are carcinogens and could cause disease in livestock[4] and possibly in humans[9]. Mammals that ingest AFB1 contaminated diets introduce into milk some amounts of the main hepatic 4-hydroxylated metabolite known as milk toxin or AFM1 that has been detected in milk and milk products[10]. Aflatoxin M1 has been detected in sera, breast milk, cord blood and maternal blood in African countries[10,11], United Arab Emirates[12], Australia and Thailand[13].

Aflatoxins often occur in crops in the field prior to harvest. Postharvest contamination can occur if crop drying is delayed or if water is allowed to exceed critical values during storage leading to mold growth[14]. Aflatoxin producing members of *Aspergillus* are common and widespread in nature [15]. They can colonize and contaminate grain before harvest or during storage. Host crops are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high humidity environment or damage from stressful conditions such as drought, a condition which lowers the barrier to entry. Pre-harvest aflatoxin contamination of peanuts and corn is favoured by high temperatures, prolonged drought conditions, and high insect activity; while post-harvest production of aflatoxin is favoured by warm temperatures and high humidity. Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed[3,16].

In recent years, enzyme linked immunosorbent assays (ELISA) have been described for aflatoxin determination. ELISA methods potentially have advantages over the other procedures because of their simplicity, sensitivity, low cost and the use of safe reagents. Extensive studies on aflatoxins in foods have validated ELISA, in comparison with very accurate, but expensive, low throughput research-oriented techniques, such as HPLC and LC/MS[1,17-19].

The study of aflatoxin contamination of foods and feeds is important because aflatoxins are toxic and carcinogenic to humans and animals. Cereal grains and related by-products have a unique importance among food commodities because they are consumed by millions of people and are considered, from a nutritional point of view, the primary source of carbohydrates for humans, breeder animals and farm livestock. The microbiological, chemical and mycotoxicological safety of cereals are considered very important for both the human and animal food chains.

A great deal of attention has been given to aflatoxin producers such as *A. flavus* and *A. parasiticus,* because AFB1 is considered by the international agency for research on cancer (IARC) to be the most carcinogenic compound produced by non-human activities. This paper employs competitive enzyme linked immunosorbent assay (ELISA) as an analytical method to examine the incidence of aflatoxin B1 in selected groundnuts and cereal grains commonly consumed in Nigeria.

Material and Methods

Sample extraction and preparation: The samples purchased from different major markets in Lagos, Nigeria were ground into powder form using roomer grinding submilling mill. Approximately 5.00 g of the sample was weighed and put in different jars and sealed. A 100 ml aliquot of 70 % (v/v) methanol was introduced into the separator funnel and the mixture was shaken for 5 minutes using orbital platform shaker model 262. The liquid portion was filtered and kept for further analysis.

Aflatoxin determination: The number of blue - bordered dilution strips required for both the samples and the standards were placed in a microwell strip holder. Equal numbers of antibody coated strip were also placed in a microwell strip holder. A multipurpose channel pipettor was used to deliver 100 ml of the conjugate into each of the blue bordered dilution well. 50 ml of the sample and the standard were placed in the dilution well containing the HRD conjugate base. The multipurpose pipettor was used to mix the sample by carefully pipetting it up and down three times. 100 ml of the mixture was immediately transferred into the antibody coated plate and incubated for 15 minutes at room temperature. The antibody coated plates were later decanted and washed five times with deionised water. A 100 ml portion of the substrate was then put into the antibody coated plate and incubated. A stop solution of 100 ml was introduced into each antibody coated plate. The microwell strips were subsequently analysed using a microwell reader. The optical density reading for each microwell was recorded. A standard curve was generated using aflatoxin concentrations in the range of 0 to 60 ng/g.

Results and Discussion

The results of the analysis are presented in table -1. A typical calibration curve is given in figure - 1 while the percent incidence is graphically illustrated in figure - 2. Preliminary investigations had shown that recovery studies of between 89.7 and 101.3% were obtained when 70% (v/v) methanol – water was employed in the extraction step. The levels of aflatoxin in wheat ranged from 4.25 to 5.17 ng/g. Maize contained 2.51 to 3.94 ng/g AFB1 contents. A range of 4.16 and 7.25 ng/g was obtained in the rice samples. Aflatoxin levels were consistently high in groundnut samples with values between 6.25 and 7.80 ng/g.

Among the 99 samples investigated, 50 were contaminated with aflatoxin (50.5 % incidence). Twenty samples of groundnut out of 24 samples (83.3% incidence) were contaminated with aflatoxin indicating that nuts were the most commonly contaminated food commodity. Our results were supported by previous reports on nuts describing nuts as the most susceptible foodstuffs to be contaminated by toxicogenic fungi producing aflatoxin[1,20,21] Aflatoxin contents of between 1.23 and 3.39 ng/g have been reported in groundnuts[14,22]. Aflatoxins are found as contaminants in various agricultural commodities. The commodities with the highest risk of aflatoxin contamination include corn, peanut, cottonseed, Brazil nut, pistachio nut, fig, spice and copra. Maize, groundnut and their products are the most important dietary sources of aflatoxin.

Many varieties of dates (*Phoenix dactylifera*) had been examined at different maturation stages for aflatoxins and aflatoxigenic Aspergillus spp. The samples were examined as fresh fruit and under simulated storage conditions of high humidity. Aflatoxins were detected in 12% of the samples and aflatoxigenic Aspergilla were detected in 40% of the varieties examined.

However, the carcinogenic potency of AFB1 is considered much lower in populations where chronic hepatitis infections are rare.

In this work, AFB1 levels in groundnuts ranged from 6.25 – 7.80 ng/g. With this high incidence of contamination the probable daily intake PDI of Nigerians could be affected by groundnut consumption even though the range was below the estimated 20 ng/g set by WHO. Currently, worldwide range of limits for AFB1 and total AF (AFT) are 1–20 ng/g and 0–35 ng/g, respectively[22]. Groundnuts are eaten raw or processed in Nigeria and its consumption cuts across all ages. The highest mean concentration of AFB1 (28.50 ng/g) was obtained in millet samples. Eighty three percent AFB1incidence with a mean concentration of 1.89 ng/g had been reported in rice[16]. The highest levels of AF tended to be found in the groundnut sauces although other ingredients cannot be ruled out as the source of AF. Aflatoxin has been

reported to be a major problem in West Africa and majority of children tested in one study in the sub region had detectable levels in their blood resulting in stunted and underweight children[23].

Ingestion of aflatoxins leads to substantial loss of productivity and degradation of meal quality in farm animals consuming contaminated feeds. AFB1 residues in eggs have been reported such that the EC set a limit for AFB1 of 20 µg/Kg for layer feed[10]. Aflatoxin can enter into the food chain mainly by ingestion through the dietary channel of humans and animals[24]. Aflatoxin contamination affects the economic value of the crops and reduces the efficiency of animal production, resulting in higher costs incurred by all sectors from production to consumption. The intake of AFB1 over a long period of time, even at very low concentration, may be highly dangerous.

It has been shown that preharvest contamination requires a drought period of 30-50 days and a mean soil temperature in the podding zone of 29-31^{o}C. Infection and aflatoxin contamination in peanuts can be related to the occurrence of soil moisture stress during pod-filling where soil temperature is optimal for A. Flavus[7]. This observation can provide the basis for a decision support system (DSS) that can be used by crop scientist to predict infection and contamination in the field in environments where aflatoxin is a serious problem.

Reduction can be achieved by good manufacturing practice and good storage practices. If the foods under investigation are to be blended with animal feeds, care must be taken since AFB1 has been shown to be a precursor of AFM1 in dairy animals [25]. Ethylene and carbon dioxide had shown promise in the battle to control aflatoxin concentration and there have not been any negative impact on the nutritive value of certain fruits[18,19]. Efforts have been made to manage AF contamination by promoting good agricultural practice (GAP) principles in the orchards and hazard analysis and critical control point (HACCP) principles in storage and processing plants. When preventive action cannot be achieved, corrective action needs to be done. Removal of AF-contaminated nuts by means of physical segregation is the most effective control measure for reducing levels of AF in a lot to an acceptable level[22]. It has been reported that roasting pistachio nuts could reduce AF contamination [26].

Aflatoxin contamination also poses a serious prenatal health hazard because it can cross the human placenta membrane and may be concentrated by the developing fetoplacental unit. Many countries have legislated minimum levels for aflatoxins in foods and worldwide regulations are in place for selected foods. The worldwide occurrence of aflatoxins in cereals is well documented, with the major contamination occurring in countries with high temperature and humidity. Corn is the most frequently contaminated cereal, whereas sorghum, rice, barley and wheat are less susceptible. Except for seasonal elevations in the contamination of corn, aflatoxin contamination of cereals rarely is a concern per se, although its frequent co-occurrence with combinations of other mycotoxins can cause serious problems. It has been reported that rice is the major contributor to the dietary intake of AFB1 in Korea and calculated that the probable daily intake of AFB1 for Koreans exceeded the estimated provisional maximum tolerable daily intake[27]. Recommendations for the control of aflatoxicosis have been proposed. The european committee regulations (ECR) establish the maximum acceptable level of total aflatoxins (AFB1, AFG1, AFB2 and AFG2) at 4 ppb, in cereals, peanuts and dried fruits either for direct human consumption, or as an ingredient in foods. In Nigeria, both human and animals feed on these investigated food commodities and chances are that unsuspecting animals may be at risk of the adverse effects found associated with aflatoxin.

Conclusion

Among the groups of toxins, AFB1 was found to be one of the most potent environmental carcinogens. Considering the high incidence of contamination (51%) and the concentration range of 2.51 - 28.50 µg/kg in these food commodities, predicted daily intake of aflatoxin in Nigeria could be affected by their consumption. In this study, aflatoxin levels were monitored in 98 samples using ELISA. In the results, 50 samples (51%) were contaminated with aflatoxin. High levels of aflatoxin in food samples emphasise the need for regular surveillance and improved control of aflatoxin levels.

ELISA technique could be applied to the monitoring of aflatoxin contamination in a large number of samples in a cost and time effective manner

Acknowledgements

The authors would like to thank the Poultry and Fish Diseases Diagnosis and Control Laboratory Lagos- Nigeria for their insightful contributions and analysis.

References

1. Chun H.S., Kim H.J., Ok. H.E., Hwang J. and Chung, D., Determination of aflatoxin levels in nuts and their products consumed in South Korea, *Food Chemistry,* **102**, 385-391 (**2007**)
2. Hall A.J. and Wild C. P., Liver cancer in low and middle income countries; prevention should target vaccination, contaminated needles and aflatoxins, *Br. Med.* J., **326**, 994-995 (**2003**)
3. Kankar A., A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab city of Iran, *Food Control,* **16,** 593-599 (**2005**)

4. CAST (Council for Agricultural Science and Technology), Mycotoxins: risks in plant, animal and human systems, Council for Agricultural Science and Technology, Ames, Iowa, Task Force Rep., **139,** (**2003**)

5. Chiavaro E., Asta C.D., Galaverna G., Biancardi, A., Gambarelli E., Dossena A. and Marchelli R., New reversed-phase liquid chromatographic method to detect aflatoxins in food and feed with cyclodextrins as fluorescence enhancers added to the eluent, *J. Chromatogr*. A, **937,** 31–40 (**2001**)

6. IARC, IARC Monograph on the evaluation of carcinogenic risk to humans, Vol. 56, Toxins derived from F. moniliform: Fumonisins B1 and B2 and fusarin C: In some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC Press, Lyons, pp. 445-466* (**1993**)

7. Bankole S.A. and O.O. Mabekoje, Occurrence of aflatoxins and fumonisins in preharvest maize from south western Nigeria, *Food Addit. Contam*., **21,** 251-255 (**2004**)

8. Kew M.C., Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. *Liver Int***., 23,** 405**-**409 **(2003**)

9. Hussein H.S., and Brasel J.M.. Toxicity, metabolism, and impact of mycotoxins on humans and animals, *Toxicology*, **167,** 101–134 (**2001**)

10. Galvano F., Ritieni A., Piva G. and Pietri. A., Mycotoxins in the human food chain. In: Mycotoxin Blue Book (D. Diaz, ed). Nottingham University Press, London, 187-224 (**2005**)

11. Jonsyn–Ellis, F.E., Aflatoxin and Ochratoxin in sera samples of School children, *J. of Nutri. and Environ Medicine*. **16**(**1**) 52-58 (**2007**)

12. Abdulrazzaq Y.M., Osman N., Yousif Z.M. and Al-Falahi S., Aflatoxin M1 in breast-milk of UAE women, *Ann. Trop. Paediatr.,* **23,** 173-179 (**2003)**

13. el-Nezami H.S., Nicoletti G., Neal G.E., Donohue D.C. and Ahokas J.T., Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand, *Food Chem. Toxicol*., **33,** 173-179 (**1995)**

14. Craufurd P.Q., Prasad P.V.V., Waliyar F. and Taheri. A., Drought, pod, pre-harvest Aspergillus infection and aflatoxin contamination on peanut in Niger, *Field Crop Research,* **98,** 20-29 (**2006)**

15. Gupta A. and Gopal M., Aflatoxin production by *Aspergillus flavus* isolates pathogenic to coconut insect pests, *World J. Microbiol. Biotechnol*., **18,** 325–331 (**2002**)

16. Ayejuyo O.O., Williams A.B. and Imafidon T.F. Ocratoxin, A burdens in Rice from Lagos Markets, Nigeria, *J of Environ. Sci. Technol*., **1**(**2**) 80-84 (**2008**)

17. Chiou, C.H., Miller, M., Wilson, D.L.,Trail, F., Linz, J.E.. Chromosomal location plays a role in regulation of aflatoxin gene expression in Aspergillus parasiticus. *Appl. Environ. Microbiol*., **68**, 306-315 (**2002**)

18. Roze L.V., Calvo A.M., Gunterus A., Beaudry R., Kall M., Linz J.E., Ethylene modulates development and toxin biosynthesis in Aspergillus possibly via an ethylene sensor-mediated signalling pathway, *J. Food Protection,* **67**, 438-447 (**2004**)

19. Gunterus A., Roze L.L., Beaudry R. and Linz J.E., Ethylene inhibits aflatoxin biosynthesis in Aspergillus parasiticus grown on peanuts, *Food Microbiology,* **24**, 658-663 (**2007**).

20. Dorner J.W., Simultaneous quantitation of *Aspergillus flavus*/*A. parasiticus* and aflatoxin in peanuts, *J. AOAC Int*., **85,** 911–916 (**2002**)

21. Escobar A. and Regueiro O.S. Determination of aflatoxin B1 in Cuba and (1990 through 1996) using an immunoenzymatic reagent kit (Aflacen), *J.Food Protection,* **65**, 219-221 (**2002**)

22. Food and Agriculture Organization of the United Nations, (FAO) Worldwide regulations for mycotoxins in food and feed in 2003, FAO Food and Nutrition Paper, No. 81, FAO, Rome, (**2004**)

23. Gong Y., Egal S., Hounsa. A., Turner P., Hall A., Cardwell K. and Wild C., determination of aflatoxin exposure in young children from Benin and Togo West Africa: the critical role of weaning, *Int. J. Epidemiol*., **32**, 556-562 (**2003**)

24. Miraglia M., Brera C. and Colatosti M., Application of biomarkers to assessment of risk to human health from exposure to mycotoxins, *Microchemical Journal*, **54**, 472–477 (**1996**)

25. Nuryono N., Agus A., Wedhastri S., Maryudani Y.B., Sigit Setyabudi F.M.C., Bohm, J., Razzazi-Fazeli, E. A limited survey of aflatoxin M1 in milk from Indonesia by ELISA, *Food Control,* **20**, 721-724 (**2009**)

26. Yazdanpanah H., Mohammadi T., Abouhossain G., and Cheraghali A.M., Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts, *Food Chem. Toxicol*., **43**, 1135–1139 (**2005**)

27. Park J.W., Kim E.K. and Kim Y.B., Estimation of the daily exposure of Koreans to aflatoxin B1 through food consumption, *Food Addit. Contam*., **21,** 70-75 (**2004**)

Table-1
Aflatoxin contents of Food Commodities (ng/g)

Sample	Tested samples	Positive samples	Percent incidence	Aflatoxin B1 concentration
Wheat unprocessed	11	2	18.2	4.25 -5.17
Maize	18	7	38.9	2.51 - 3.94
Sorghum	10	3	30.0	5.20 - 6.25
Rice	20	12	60.0	4.16 -7.25
Groundnut	24	20	83.3	6.25 – 7.80
Millet	16	6	37.5	4.18 – 28.50
Summary	99	50	50.5	2.51 -28.50

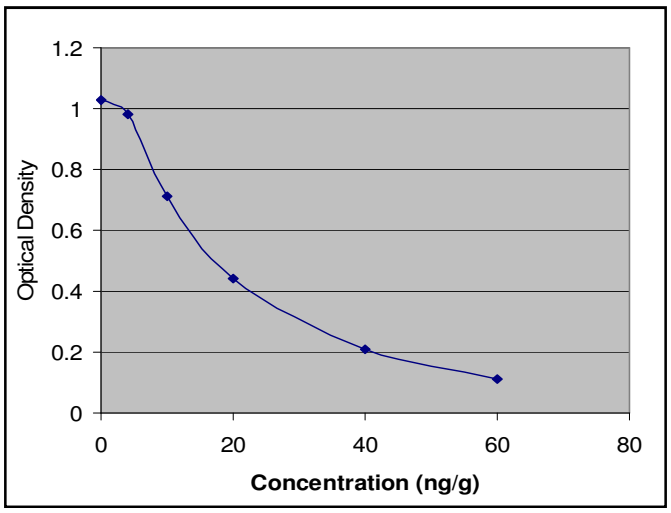

Figure -1
Typical calibration curve

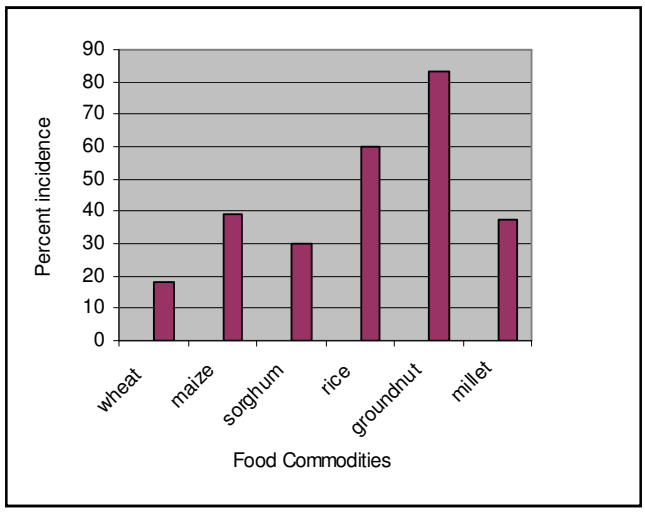

Figure-2
Incidence of afflatoxin in foods