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RESEARCH ARTICLE

Determination of Aflatoxins in Sesame, Rice, Millet and Acha from Nigeria using HPLC

MAKUN HUSSAINI ANTHONY[1*], APEH DANIEL OJOCHENEMI[1,2], ADEYEMI HENRY RINDE YEMI[1], NAGAGO TAHIR[1], OKEKE JOHNBOSCO OKECHUKWU[1], MUSTAPHA AMINA SAIDU[1] and OYINLOYE BUKUNMI AYOBAMI[1]

[1]Biochemistry Department, Federal University of Technology Minna, Niger State, Nigeria
[2]Family Health International (FHI360) Country Office, Abuja, Nigeria
danapeh@gmail.com

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Abstract: A hundred and twenty (120) Nigerian food commodities including; rice (15), madidi (rice product) (15), millet (15), fura (millet dough) (15), sesame (30) and fonio (30) were collected, subjected to aflatoxin extraction and clean up procedures and analyzed quantitatively for aflatoxins using High performance liquid chromatography. Five of the rice samples contained aflatoxin B_1 within the range of 37.26-113.2 µg/kg, for madidi samples, 7 contained AFB_1 within the range of 0.20-112.5µg/kg, 3 contained AFB_2 within the range of 0.95-18.40 µg/kg while 6.88 µg/kg and 1.60 µg/kg of the green fluorescing aflatoxins 1 and 2 respectively was found in one sample each. Analysis of sesame samples showed that eight contained AFB_1 within the range of 14.71-140.9 µg/kg, while one sample contained 2.61 µg/kg of aflatoxin G_1. The millet, millet dough and fonio samples all tested negative for the presence of aflatoxins indicating some degree of resistance. All contaminated rice and sesame samples were at ranges above the EU and Nigerian legislated limits for AFB_1 and aflatoxins in food. This portrays associated health consequence to consumers and negative impact on trade of these commodities both locally and internationally.

Keywords: Aflatoxin, Rice, Millet, Fonio, Sesame

Introduction

Several episodes of aflatoxicosis has been put to record in human history, notably among which are the Western India outbreak of 1974 with 106 deaths of indigenous people whose staple food was maize, and the rural Kenya episode of 2004 which claimed the life of 125 natives of which aflatoxin-contaminated home grown maize was also responsible[1-2]. Apart from maize, aflatoxin have been reported in peanuts, rice, bread, cooked meats, sorghum, barley[3-7]and human milk as a function of the dietary exposure of the mother to AFB_1[8-9]. Aflatoxin are secondary metabolites of fungi belonging to the genera *Aspergillus* and it has been associated with the toxigenic members of the *A. flavus, A. parasiticus, A. nomious, A. tamarii* and *A. ochraceoroseus*[10-11]. Of the four aflatoxin subtypes, AFB_1 is the most

important in terms of occurrence and toxicity[12-13]. Aflatoxin have been associated with health effects including liver cancer, liver and kidney diseases, immunologic suppression, growth impairment among other disease conditions[14-17].

Lack of awareness, management practices and toxin data necessary for legislative purposes, were possible causes of the Indian and Kenyan outbreaks until these lapses are fixed, a repeat of history is anticipated even in other part of the world. This should be taken seriously as human life is always involved directly or indirectly. Also worthy of attention is the current trend in global warming, which continuously provide warmer temperatures likely to provide optimum temperature which favours the growth of aflatoxigenic fungi[18]. Such temperature range as found in the tropical and subtropical regions together with relative humidity of over 70% significantly favours the growth of moulds. Aflatoxin contamination can occur during crop development when the crop is either damaged (*e.g.*, by insects) or stressed by heat and drought and after maturation when the crop is exposed to high moisture and high temperature either before harvest or in storage[19-20]. The death rates in the reported episodes above were directly related to high dependence on maize as the major staple in those regions. Over time, dependence on maize has subsided due to the recognition of the nutritional value of other staples such as rice, millet and fonio. Sesame is also consumed widely in different forms. Also this energy sources are less susceptible to fungal and aflatoxin[21].

Fonio locally referred to as "acha" is a very old African crop cultivated for its nutritional attributes including high amino acids content particularly methionine which is twice that in egg protein[22] and cystine which supply sulfur and other compounds required by the body for normal metabolism and growth [23]. Fonio is also rich in phenylalanine, another essential amino acid[24] and can therefore potentially replace legumes to complement standard diets. Fonio is one of the grains with very high magnesium, zinc and manganese levels. Comparatively, it is significantly richer in vitamins B_1 and B_2, calcium and phosphorous than white rice[25]. Out of the total world production of 583,882 metric tonnes, Nigeria produced 90,000 metric tonnes in the 2012 fiscal year amounting to 15.41%, making her the world second largest producer after Guinea, an improvement over the previous 80,000 metric tonnes it produced in 2011. Consumption of this grain is also high locally as it is consumed as porridge.

Sesame (*Sesamum indicum L.*) commonly known as beniseed is one of the oil seeds cultivated in Nigeria, being the eight and fifth largest sesame producer in the world and in Africa respectively. Nigeria contributes 158,000 metric tonnes in a world total of 4,167,150 metric tonnes. From its introduction after the Second World War, it was regarded as a crop of insignificant importance compared to groundnut and other cash crops until about 1974 when it became one of the major cash crops in many Northern Nigerian states[26]. Sesame has continued to gain increased recognition due to the presence of omega-3-fatty acid, essential oils as well as natural antioxidant sesame in that both prevent aging and is vital to liver cell production. Dietary supplement of 40 g per day lowers serum total cholesterol and low density lipoprotein cholesterol and consequently protects hypercholesterolemic patients from atherosclerosis[27,28]. Sesame oil has been shown to ameliorate cough in children[29]. It is also rich in protein with amino acid profile similar to soybean. These sterling attributes recently discovered, are stimulating interest in the production of the crop. Owing to its previous status as a minor crop, there has been little research efforts on the crop so far. From Nigeria, sesame is exported majorly as seed and the destination is majorly Asia ranking amongst the top 5 exported products. Sesame seed has the risk of contamination during storage by mycotoxins especially the ubiquitous and hepatotoxic aflatoxins, which are produced when

seeds and nuts are kept under conditions that favour the development of these fungi. Contamination of sesame seed along the supply chain is of major concern for public health and trade.

Eighty countries produced 25, 597, 550 metric tonnes of millet in 2012, the contribution of Nigeria was 1,000,100 metric tonnes making it the sixth largest producer after India, Niger, Mali, China and Burkina Faso in the year under review. Millet remains a key source for food security and energy for about 250 million people in sub-Saharan Africa. It is consumed after it has been processed into various forms of meal, biscuit, gruel, cake pap and porridge[30]. The susceptibility of millet to fungal growth and mycotoxin contamination has been documented[31,32]. The limited mycotoxin research on millet in Africa is understandable as it is one of the 'lost crops of Africa' neither is it an export crop. However, its protein and vitamin contents, resistance to drought and resistance to mycotoxin contamination has brought this African traditional crop to the front burner of research worldwide because it is anticipated to boost food production in poverty and drought stricken regions of the world as well as reduce the economic and health risks to food spoilage organisms and toxins[33].

Rice (*Oryza sativa*) is a major staple in many part of the world and has been patronized at world levels with a large market. The world total rice production in 2012 was 718,345,380 metric tonnes of which, Nigeria with status as the second world leading rice importer, produced about 4,833,000 metric tonnes making her the seventeenth of over one hundred and seventeen producing countries. Rice is grown approximately on 3.7 million hectares in Nigeria, covering 10.6% of the 35 million hectares of land under cultivation, out of a total arable land area of 70 million hectares[34]. In terms of calorie rice was the fourth most important crop in Nigeria between 2000 and 2007 after sorghum, millet and cassava[34.]

Aflatoxin contamination constitutes a major setback to export trade in grains and cereals proceeding from Africa, as a result it poses a challenge to food security in areas that are dependent on these staples. This is due to available legislative limits provided by different countries. Considering their health and economic implications, there is therefore the need to elucidate the mycotoxin profile of these crops within regions where they are produced and marketed, with a view to generating incidence data which can be used to proffer intervention strategies. The objective of this study therefore is to determine the aflatoxin content of sesame, millet, fonio, rice and some of their products produced and marketed in Northern Nigeria.

Experimental

Sampling was conducted between the months of June and July, 2012. About 500 g each of 120 food samples were collected thus; Fifteen (15) samples each of rice and madidi (rice product) were randomly collected from various locations in Nasarawa state. Thirty (30) samples each of fonio and sesame seeds were collected from four markets within Minna, Niger state, 15 samples each of millet and millet dough were also collected from Kontagora in Niger state. All samples were sealed in plastic bottles and stored at -20^{o}C in deep freezer before analysis.

Extraction of Aflatoxin and Clean-up

The extraction method of Ehrlich *et al.*[35] was used. This method uses methylene chloride and phosphoric acid for the simultaneous extraction of aflatoxin B_1, B_2, G_1, G_2 and OTA, which is then subjected to specific clean up procedure for aflatoxins as elaborated thus; Twenty-five grams (25 g) portion of pulverized sample/paste equivalent was weighed/poured into 500 mL Erlenmeyer flask. One molar phosphoric acid (12.5 mL) and methylene chloride

(125 mL) was added. The flask was covered with a stopper and shaken for 30 minutes. The content was then filtered on a funnel fitted with Whatmann No. 1 filter paper. About 120 mL of the filtrate was collected and from this, 50 mL aliquot each was placed in separate 100 mL Erlenmeyer flasks with glass stoppers ready for clean-up.

During clean-up, a separating column was set with glass wool, into which 150 mL of methylene chloride (CH_2Cl_2) was poured and drained halfway through one scoop of anhydrous sodium sulphite (Na_2SO_4) on the filter paper. The remaining methylene chloride was drained then silica gel was added into the column and 80 mL methylene chloride was poured in and allowed to settle before it was drained half way. Three scoops of sodium sulphite were added and the remaining half of the methylene chloride was drained completely. Filtrate sample (25 mL) was added and drained completely, 65 mL of *n*-hexane was added and drained, 65 mL of petroleum ether was also added and drained. 65 mL of solvent consisting of petroleum ether, methanol and water in the ratio (96:3:1) was added and drained into a clean beaker. This portion now containing the toxin was evaporated to about 2 mL and finally dispensed in an amber bottle and refrigerated for HPLC analysis.

High Performance Liquid Chromatography

Agilent technologies 1200 series HPLC fitted with Octadecylsilyl groups (ODS), (4.6-150mm-5um) column set at ambient temperature with mobile phase being acetonitrile:water:methanol (10:50:40 v/v) was used. The machine flow rate was set at 0.8 mL/min and the sample Injection volume was 20 µL. The detection limit of the machine with regards to the aflatoxin was 0.1µg/kg and the recoveries for each of the toxins were greater than 85%.

Statistical analysis

All the analytical data generated were subjected to statistical analysis using SPSS (version 16.0) software. The statistical level of significance was fixed at $P < 0.05$ (95%)

Results and Discussion

Aflatoxin producing fungi such as *Aspergillus flavus* thrive on several food commodities in Nigeria[7,18,36,37]. In sesame samples, low incidence (8/30) of aflatoxin was observed with high concentration in positive samples (Table 1). All contaminated sesame samples were at levels above the EU maximum limits of 2 µg/kg for aflatoxin B_1 and 4 µg/kg for total aflatoxin. The EU limit has been adopted and is currently used as the standard in Nigeria. However, the Nigerian limit was 10 µg/kg for total aflatoxin before now. Aflatoxin B_1, was found at Mean±SEM value of 18.59±6.57 µg/kg. Only one sample was contaminated with aflatoxin G_1 at 2.61 µg/kg. Thus the ratio of occurrence is 1:0.14 for AFB_1:AFG_1. This presents a slight deviation from the reported natural occurrence of aflatoxin being 1.0: 0.1: 0.3: 0.03 for AFB_1, AFB_2, AFG_1, AFG_2 respectively[39,40]. Studies on aflatoxin in sesame in Nigeria includes that of Ezekiel *et al*.,[41] who demonstrated that sesame is less susceptible to aflatoxin presenting an incidence of 0/17 and that of Mbah and Akuesi[18] who incubated two species of sesame with *Aspergillus flavus* and found that only 4 out of 60 samples were contaminated with about 25 ppb of aflatoxin B_1 after 20 days of incubation. Both studies indicate low susceptibility of sesame to aflatoxin, despite the fact that the high oil content of sesame is perceived to make it a good substrate for fungal growth[40]. Our work further demonstrates low aflatoxin incidence (8/30) however at unsafe levels. Factors responsible for this difference could be attributed to difference in geographical region of sampling, as both authors[18,41] sampled from Plateau state Nigeria. Plateau state is known for a relatively low temperature and

high relative humidity all year round when compared to Niger state were we sampled. Contamination could also result from poor handling and unwholesome practices along the post-production chain.

Table 1. Aflatoxin concentrations in samples of dry sesame seed, rice and rice product (Madidi), fonio, millet and millet dough (fura) (µg/kg) and their safety status

Sample Type/ Frequency	Sample Code	AFB_1 µg/kg	AFB_2 µg/kg	AFG_1 µg/kg	AFG_2 µg/kg	Total AF	Safety Status
Rice (5/15)	R1	37.26				37.26	Unsafe
	R4	40.24				40.24	Unsafe
	R7	113.20				113.2	Unsafe
	R13	75.38				75.38	Unsafe
	R15	112.40				112.4	Unsafe
	Range	37.26-113.20					
	Mean ± SD	75.38±37.04					
Madidi (8/15)	M1	1.00	1.40	6.88	1.60	10.88	Unsafe
	M3	1.40				1.40	
	M7	8.30				8.30	Unsafe
	M8	0.20				0.20	safe
	M9		0.95			0.95	safe
	M11	1.40				1.40	safe
	M14	125.60	18.40			144.0	Unsafe
	M15	2.84				2.84	Unsafe
	Range	0.2-125.60	0.95-18.4				
	Mean ± SD	20.11±46.60	6.92±9.95				
Dry Sesame seed (8/30)	SS1	40.00		2.61		42.61	Unsafe
	SS2	14.71				14.71	Unsafe
	SS3	49.39				49.39	Unsafe
	SS4	59.98				59.98	Unsafe
	SS5	140.90				140.9	Unsafe
	SS6	49.04				49.04	Unsafe
	SS7	111.44				111.4	Unsafe
	SS8	92.26				92.26	Unsafe
	Range	14.71-140.90					
	Mean ± SD	69.72±41.68					
Millet (0/15)	M1-M15	ND	ND	ND	ND	NA	Safe
Millet dough (fura) (0/15)	MD1-MD15	ND	ND	ND	ND	NA	Safe
Fonio (0/30)	F1-F15	ND	ND	ND	ND	NA	Safe

ND – not detected; NA – not applicable

Information on aflatoxin contamination of rice worldwide including few from some parts of Nigeria are available[32,42,43] with some unpublished. However, to the best of our knowledge in Nasarawa state of Nigeria, no study on aflatoxin in rice has been carried out neither has any work been done on aflatoxin in madidi (rice product). In this study, rice

samples tested positive for aflatoxins B_1, B_2, G_1 and G_2 at low incidence; 5/15 for rice and 7/15 for madidi. Generally, a lower aflatoxin concentration was found in madidi when compared to rice. This drop in aflatoxin may be due to the processes involved in the production of madidi; soaking and heating. Soaking and subsequent decantation has been shown to reduce aflatoxin levels[44,45]. Aflatoxin B_2 was also found in 3/15 madidi samples at concentrations ranging between 0.95-18.40 µg/kg. AFG_1 and AFG_2 were present in one sample each of madidi at concentrations of 6.88 µg/kg and 1.60 µg/kg respectively. The early work of Opadokun[40] showed a low incidence (13/ 279) of AFB_1 in rice samples with mean value of 5 µg/kg lower than what was found in this work. Makun *et al.*[43] using a very sensitive method of analysis, found each of AFB_1, B_2, G_1 and G_2 in at least 19 of 21 rice samples from Niger state. Other works especially on fungi profile have also demonstrated risk of aflatoxin contamination in rice[43].

All the fonio, millet and millet dough (fura) samples analyzed showed noaflatoxin contamination, probably because they are present below detectable limit despite the sensitivity of the method used. Available reports indicate either low incidence and/or concentrations of aflatoxin in fonio, among these are Ezekiel *et al.*[41] who had a high incidence (81%) but low levels (0.08-1.4 µg/kg) below the EU maximum limit and Gbodi *et al.*[46] who reported low incidence of 4/24 and 2/24 for both AFB_1 and AFB_2 at range of 0-20 µg/kg and 0-12 µg/kg respectively of aflatoxin in fonio. In Nigeria, aflatoxin contamination of millet has also been reported however at low incidence which were found mostly at unsafe concentrations [42,43, 47]. Other studies on aflatoxin in millet outside Nigeria include the works of Mishra and Daradiyhar[48] and Wilson *et al.* [49] who also reported aflatoxin B_1 at unsafe levels in stored millet, cooked millet and pearl millet,with highest levels of toxin in stored millet, thus reiterating the importance of providing proper storage conditions which will not favour the growth of fungi and mycotoxin production. Part of the reason for the low levels of aflatoxin earlier reported in millet and fonio as well as the low susceptibility found in this work is not unrelated to their phytochemical compositions. Viswanatha *et al.*[50] showed that phenolic compounds in the seed coat of millet are active against fungi giving millet some antifungal attributes. It was also well stated that phenolic including tannins which are present in this grains are involved in grain resistance to fungi attack [51]. Also, the tiny nature of millet and fonio attributes them small surface area for mould infestation this in turn, reduces their susceptibility to mycotoxins significantly[52].

Exposure to aflatoxin in West Africa is widespread, blood tests have shown that very high percentage of West Africans are exposed to aflatoxin[53]. In a study carried out in the Gambia, Guinea Conakry, Nigeria and Senegal, over 98% of subjects tested positive to aflatoxin markers[53]. This is attributed to high dependence on aflatoxin susceptible foods including but not strictly restricted to maize, sorghum and rice in this region. Aflatoxin is a very powerful hepatocarcinogen, and naturally occurring mixtures of aflatoxins as found in some sesame and madidi samples in our work has been identified as a class 1 human carcinogen[8].

The impact of the presence of AF in rice and sesame on health and trade in Nigeria cannot be overstated, this is because of the high consumption rate of both products among nationals and also due to international trade demand of this products. On average between the fiscal year 2000-2007 rice was the 4th most important crop in terms of calories following sorghum, millet and cassavain Nigeria. Being both a food and a cash crop for local farmers, it contributes to small holders revenues in the main producing areas. WARDA estimates that per capita rice consumption in Nigeria has nearly doubled between the 1980s and 2006, growing from 15.4 kg/year to 25.4 kg/year[54] this figure tells of the impending danger in

continuous consumption of aflatoxin infested grains. In 2011 alone, Nigeria exported about 166 tonnes of rice valued at $3000 and 124,700 tonnes of sesame valued at $148,613,000 this figure especially for sesame can be improved on if fungi and aflatoxin contamination is reduced and managed properly.

Based on our findings, we recommend the diversification of diet such that over dependence on crops that are highly susceptible to aflatoxin is reduced. Fonio and millet should be incorporated as energy sources especially in the regions were we sampled. This will however require proper orientation and awareness campaign on the impending danger of over dependence on the major staples (maize and sorghum) of the region which have been shown to be susceptible to fungi that produce aflatoxin. This if practiced will to a great extent reduce aflatoxin ingestion and associated health hazards.

Conclusion

Five of fifteen rice samples contained AFB_1 at levels above the EU and Nigerian legislative limits. Seven madidi samples contained AFB_1 while three contained AFB_2, one each contained AFG_1 and AFG_2 respectively. The trend in the study shows a general reduction in aflatoxin concentration from rice grain to rice product (madidi) as a result of processing. Eight of thirty sesame samples contained AFB_1 also above the safe level, while one sample contained AFG_1. The incidence was low for both crops but the concentrations were mostly above the permitted legislative limits. Considering the aforementioned, it is therefore, needful to employ good agricultural practices (GAPs) both before and after crop harvesting as well as lay more emphasis on proper monitoring activities. Also the fact that levels of the toxins found were at concern levels should trigger enforcement of regulations by concerned national bodies that are responsible for setting standards and those responsible for enforcing set standards.

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