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Natural occurrence of ochratoxin A in some marketed Nigerian foods

Hussaini Anthony Makun [a,*], A.L. Adeniran [b], Simeon Chidawa Mailafiya [a], Ifedapo Solomon Ayanda [a], Afeez Temitayo Mudashiru [a], Uzochukwu Jeffrey Ojukwu [a], Abel Sunday Jagaba [a], Zakari Usman [a], Danlami Adam Salihu [a]

[a] Department of Biochemistry, Federal University of Technology, PMB 65, Bosso Campus, Minna, Niger State, Nigeria
[b] Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Abuja, Nigeria

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ABSTRACT

A total of one hundred and nine samples of maize (*Zea mays*) (17), millet (*Pennisetum* spp) (18), guinea corn (*Sorghum*) (17), acha (*Digitaria exilis stapf*) (20),sesame (*Sesamum indicum*) (19) and fermented cassava (*Manihot esculenta*) flakes (garri) (18) from markets located in Minna and its environs were analysed for ochratoxin A (OTA) by High Pressure Liquid Chromatography (HPLC). OTA was detected in 98.2% of the samples. The levels found were maize (0–139.2 μg/kg), millet (10.20–46.57 μg/kg), guinea corn (0–29.50 μg/kg), sesame seeds (1.90–15.66 μg/kg), acha (1.38–23.90 μg/kg) and garri (3.28 –22.73 μg/kg). Maize had the highest level of OTA with "acha" having the lowest content of the toxin. The OTA levels found in marketed food and feed commodities which were mostly (74.3%) above 5 μg/kg, the European Union standard raise public health concern. The study is the first report of OTA contamination of acha, sesame seed and garri in Nigeria and possibly in Africa.

1. Introduction

The main sources of nutrition in the world are cereals, and roots and tubers. The other complementary nutritional sources include animal products, vegetables, fruits, etc. Cereals are the more significant of the two because they are staple foods for two third of the earth's population, providing 85% and 10% of the world's food energy and protein intake respectively (Shewry, 2007). Roots and tubers on the other hand are basic diets for about a billion people in the developing countries, accounting for 40% of food eaten by half the population of Sub Saharan Africa (FAOSTAT, 2009). The same FAO statistics show that in Africa, cereals and, roots and tubers contribute 46% and 20% of the total energy intake respectively. While maize, rice, wheat, Sorghum and millet are the major cereals eaten, cassava, potatoes, yam and taro form the bulk of the roots and tubers consumed worldwide. These food crops are not only serving nutritional needs but are also being used for industrial and consequently economic purposes such as biofuel production.

In Nigeria, of the nine staple food crops, maize and cassava are the first and second most frequently consumed crops with sorghum being the seventh after rice, cowpea, groundnut and yam. Plantain and soybean are the last two respectively (Maziya-Dixon et al., 2004). Maize as cornmeal constitutes a staple in the country. It is also eaten in boiled and roasted forms and as popcorn. It is widely grown for animal feeds. Cassava is eaten mainly as garri. In the production of garri, cassava tubers are peeled, washed and grated or crushed to produce a mash. The mash is placed in a porous bag and allowed to ferment for one or two days, while weights are placed on the bag to press the water out. It is then sieved (or sifted) and roasted by heating in a bowl. The resulting dry granular garri can be stored for long periods. Garri can be taken as stiff dough ('eba'), or as snacks or light meal (soaked in cold water).

Sorghum like maize is commonly taken as meal and has increasing relevance in brewing of local beers ("burukutu" and "pito"). Millet is commonly consumed as pap, porridge, local cake ("masa"), millet meal ("tuwo"), gruel-like drink ("kunu – zaki"), and "fura" in the Northern Nigeria where it is cultivated. Sesame seed is an oil-rich seed that is used in sweet confections, bread, eaten as snacks and used as soup thickener in most parts of Nigeria. Acha commonly called hungry rice or white fonio is used as porridge and for making gruel-like drink called "kunnu" in Northern Nigeria.

According to FAOSTAT (2010), maize, cassava, sorghum and millet are amongst the six most cultivated and consumed crops in Nigeria. Sesame and fonio are also substantially grown and eaten in

* Corresponding author.
E-mail address: hussainimakun@yahoo.com (H.A. Makun).

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the country. The latest FAO statistics on crop cultivation area and production is for the year 2010 and it reports that Nigeria produced 7.3 million tonnes (MT) of maize in the year under review making it the 14th largest producer of the grain amongst the world's 163 maize producing countries. The same FAO figures showed that Nigeria is the world largest producer of cassava (37.5 MT) of the 101 producing countries. It also harvested ≥8% (4.7 MT) of the total world sorghum produced (55.6 MT) in 2012 ranking it the 4th world leading producer of the cereal in 2012 after USA, India and Mexico. The data also showed that 4.1 MT, 0.1 MT and 45,200 tonnes of millet, sesame and hungry rice respectively were produced and consumed in the country making it the 2nd (out of 82 producing countries) and 7th (out of 70 countries) top millet and sesame producing countries in the world. Niger State, the study area cultivates and harvests between 10% and 20% of the national cereal and tuber produced by Nigeria (Akpobasah, Okpanachi, Nwosu, & Abara, 2004).

Ochratoxin A (OTA) is a toxic secondary metabolite of over ten fungi but is mainly produced in foods by *Aspergillus ochraceus* in the tropics and *Penicillium verrucosum* in the temperate regions (Kumar, Basu, & Rajendran, 2008). It is a potent nephrotoxin that causes kidney and liver impairment in animals (especially in pigs) and human beings (Hussein & Brasel, 2001). It has been associated with endemic Balkan nephropathy that is often accompanied by upper urinary tract urothelial cancer (Grollman & Jelaković, 2007; Hult, Piestina, Habazin-Novak, Radic, & Ceovic, 1982). Ochratoxin A is potentially carcinogenic to humans and belongs to group 2B list of carcinogens (IARC, 1976). The mycotoxin has been shown to be weakly mutagenic, possibly by induction of oxidative DNA damage (Palma, Cinelli, Sapora, Wilson, & Dogliotti, 2007).

The susceptibility of cereals and cereal based products, nuts, coffee beans, swine products, wine, beer and other beverages to OTA contamination worldwide is documented (Duarte, Pena, & Lino, 2010) Kumar et al., 2008; Njobeh, Dutton, Chuturgoon, et al., 2010). In a few reports the toxin has been shown as contaminant of maize (Adebajo, Idowu, & Adesanya, 1994; Gbodi, Nwude, Aliu, & Ikediobi, 1986) and maize based weaning food (Oyelami, Maxwell, & Adeoba, 1996), sorghum (Elegbede, West, & Audu, 1982; Makun, Gbodi, Akanya, Salako, & Ogbadu, 2009), rice (Makun, Dutton, Njobeh, Mwanza, & Kabiru, 2011; Makun, Gbodi, Akanya, Salako, & Ogbadu, 2007), kolanut and cocoa beans (Njobeh, Dutton, & Makun, 2010) and tiger nut (Adebajo, 1993) in Nigeria. Except for few works (Makun et al., 2007, 2009, 2011), information on the presence of this nephrotoxin in cereals in Niger State, a food basket of Nigeria which is a world leading producer, consumer and exporter of cereals and other food products is scarce. It is pertinent to also note that investigations on OTA are concentrated on cocoa beans, maize and sorghum with little or no data on its contamination of equally highly cultivated and consumed agricultural products such as millet, cassava products, sesame and acha. Hence, this study attempts to bring up-to-date current status of OTA contamination of marketed samples of maize, sorghum, millet, acha, sesame and garri in Niger State, an extremely agrarian region of the country.

2. Materials and methods

2.1. Sampling

One hundred and nine samples of six different marketed human food commodities were randomly collected from 109 foodstuff vendors from twenty markets in Minna, Paiko and Kaffin Koro towns of Niger State, Nigeria in 2010. About 0.5 kg of each sample was collected and put in sealed plastic bottles and stored at −20 °C in the deep freezer until used for analysis.

2.2. Extraction of ochratoxin A

Ochratoxin A was extracted using AOAC official method (Ehrlich & Lee, 1984). In this method, 50 g of pulverized samples were weighed into 500 ml Erlenmeyer flask and 25 ml 1M-phosphoric acid and 250 ml of methylene chloride were added. The flask was shaken for 30 min using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 200 ml of the filtrate was collected. From this, 50 ml aliquots were placed in separate 100 ml Erlenmeyer flasks with glass stoppers and subjected to specific clean up for OTA assay as follows. Bicarbonate solution (70 ml) was added and shaken. After phase separation, the lower methylene chloride layer was drained into a 250 ml separatory funnel with addition of 35 ml sodium bicarbonate solution. The lower methylene chloride layer was discarded and the aqueous layer acidified to PH 3.5 with sulphuric acid. The acidified layer was transferred into a second separatory funnel and OTA further extracted into 50 ml of methylene chloride which was drained through sodium sulphate into a beaker. After two more rinses with 50 ml each of methylene chloride, the pooled extract was evaporated to dryness and transferred into 4 ml amber sample bottle, and stored at −20 °C in the deep freezer until used for analysis. The dry film was reconstituted with 200 μL mobile phase (Acetonitrile:water:acetic acid (50:48:2)) for HPLC analysis.

2.3. High pressure liquid chromatographic technique

OTA was quantified on Cecil 1100 series HPLC with UV detection as described by Engstrom, Richard and Cysewski (1977) at wavelength of 254 nm. The altraspher ODS column, 4.6 mm × 25 cm was used at ambient temperature of 25 °C. Acetonitrile : water and acetic acid in ratio 50:48:2 respectively was used as mobile phase at flow rate of 1 ml/min. The injection volume was 60 μL. Calibration curve (Fig. 1) with a correlation factor of 0.925 was determined

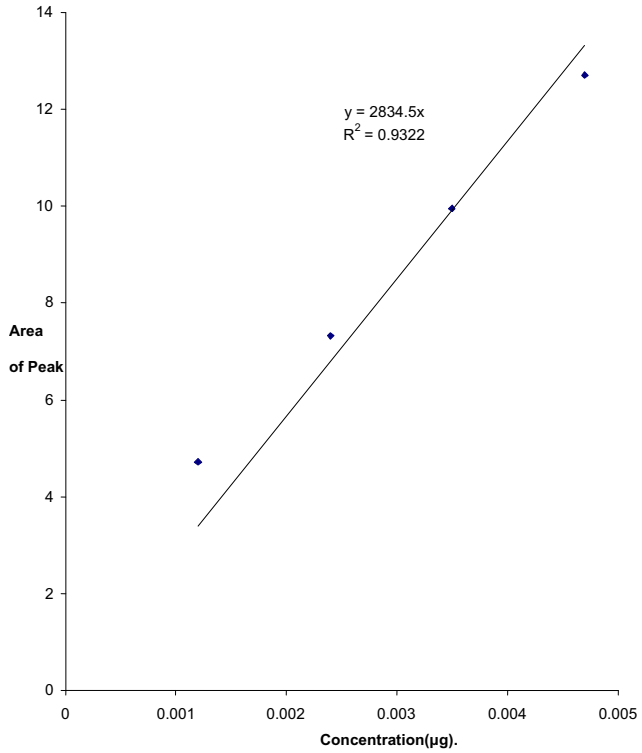

Fig. 1. Calibration curve for ochratoxin A standard.

using series of dilutions containing 0.023 μg/ml, 0.018 μg/ml, 0.014 μg/ml, 0.009 μg/ml and 0.004 μg/ml. The retention time for OTA was 1.11 min (Figs. 2 and 3) while the detection limit of the machine with regards to the toxin was 0.001 μg. 10 μg/ml of OTA was spiked in 3 samples of each food commodity and recovery rates determined. The results are shown on Table 1. The observed recoveries indicate that the sensitivity and reliability of the methods employed were sufficient for evaluation of OTA in foodstuffs.

2.4. Statistical analysis

All the analytical data generated were subjected to statistical analysis using SPSS (version 10.0) software. The statistical level of significance was fixed at $P < 0.05$ (95%).

3. Results and discussion

The volume of literature on OTA in Nigeria is limited. The present work presents for the first time the ochratoxin A profile of millet, sesame, 'acha' and 'garri' in Niger State. The data generated for maize and sorghum will complement the few available ones from Nigeria which were in predominantly non representative mouldy samples (Elegbede et al., 1982; Makun et al., 2009). Table 2 summarizes the incidence and concentration of OTA in six Nigerian food commodities. The concentrations reported were adjusted based on recovery rates obtained. The survey has shown OTA as contaminants of all the studied products with the highest concentration in maize followed by millet, sorghum, sesame, 'garri' and 'acha' in decreasing order. This variation in concentrations and therefore vulnerability to the toxin is grain size dependent with OTA levels reducing with decreasing size of the grain i.e maize > millet > sorghum > sesame > acha. Small, compact grains (in this case acha, sesame, sorghum and millet) and those encapsulated in hard seed coats (beans and soybeans) are less susceptible to fungal infection and mycotoxin formation than larger grains such as maize with less formidable coats (Stössel, 1986), hence the noted seed size dependent differences. The exception to this trend in this study is the observed higher OTA levels in millet than in sorghum which is a bigger grain and this might not be unconnected with the greater amount of fungicidal principles; phenols and tannins, that provide resistance to mould infestation and mycotoxin formation

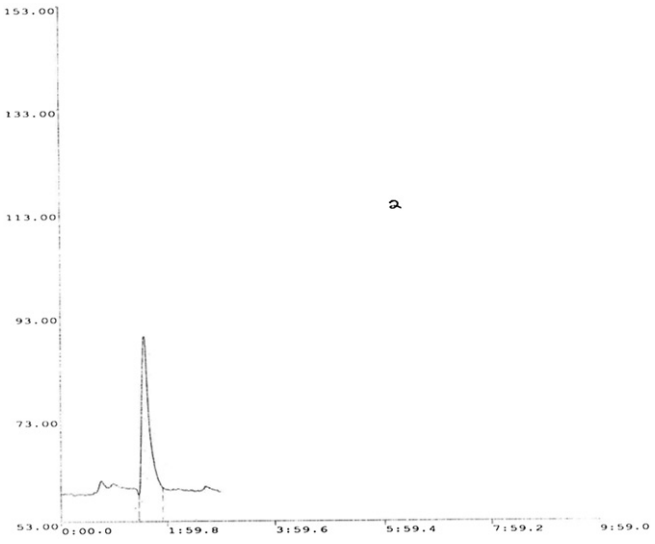

Fig. 2. Chromatogram of ochratoxin standard.

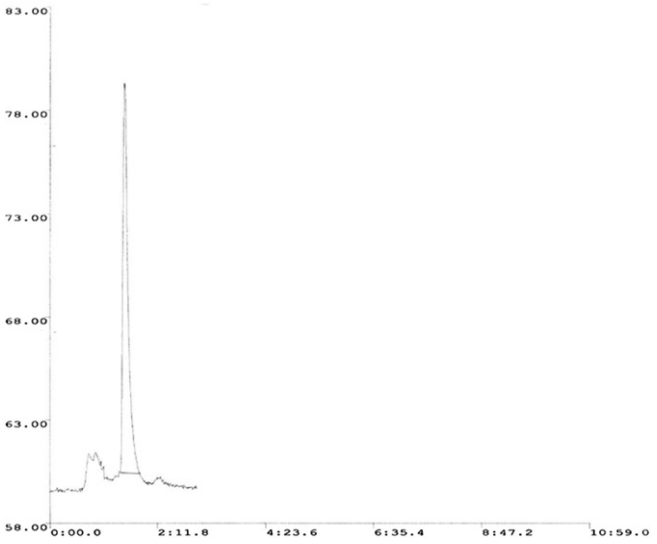

Fig. 3. Chromatogram of positive sample containing ochratoxin A.

(Audilakshmi, Stenhouse, Reddy, & Prasad, 1999), in sorghum than millet (Dykes & Rooney, 2006). It is not surprising that garri has the least OTA after 'acha' because its raw material, cassava is not susceptible to mycotoxin contamination (Essono et al., 2009) and more so it has undergone milling and fermentation which are procedures that are known to reduce mycotoxins in processed products (Hell & Mutegi, 2011).

Maize is the most important ingredient of many foods in Africa. Being a major component of weaning foods and animal feeds makes its contamination by OTA a serious food safety issue. The current study revealed high prevalence (94.1%) of OTA in maize from Niger State and 14 of the 17 samples analysed had OTA contents above the EU regulatory limit of 5 μg/kg for raw cereal grains (CEC, 2006). Previously Gbodi et al. (1986) had found the toxin in maize in Plateau State, Nigeria at 75% frequency at concentration range of 0–150 μg/kg and mean value of 23.75 μg/kg while Adebajo et al. (1994) detected OTA in 1, 4 and 1 samples of 20 samples each of corn, corn cake and corn roll snack at mean concentrations of 5, 38 and 10 μg/kg respectively in south western states of Nigeria. Oyelami et al. (1996) observed 8.3% prevalence at concentrations of between 0.142 μg/kg and 6.516 μg/kg in maize based weaning food in the South West of the country. Using LC/MS/MS, Ezekiel, Bandyopadhyay, Sulyok, Warth, and Krska (2012) found OTA in 34% of 59 poultry feeds collected from 17 states of the country at mean and range concentrations of 10 ± 6 μg/kg and 4–26 μg/kg. The feeds had the following composition maize, wheat offal, groundnut cake and soybean. Technological processing including milling which removes parts of seeds containing mycotoxins results in lower OTA prevalence and amount in non whole grain cereal samples (Juan, Moltó, Lino, & Mañes, 2008) and processed cereals (Park, Chung, & Kim, 2005) than whole-grain cereal samples. This explains the higher frequency and concentration of OTA observed in raw maize in this study and that of Gbodi et al. (1986) than in processed products as shown by Adebajo et al. (1994), Oyelami et al. (1996) and Ezekiel, Bandyopadhyay, et al. (2012). The incidence of the toxin in maize as shown in this work is comparable to the prevalence and therefore the high vulnerability of the grain to OTA as demonstrated worldwide (Duarte et al., 2010; WHO, 1990). The nutrient composition of maize; adequate carbohydrates as energy source, trace elements, organic and inorganic nitrogen (particularly glutamic acid which is incorporated into OTA) (González-Osnaya, Soriano, Moltó, & Mañes

Table 1
HPLC recovery of ochratoxin A for maize, millet, sorghum, sesame, acha and garri.

Food commodity	Maize	Millet	Sorghum	Sesame	Acha	Garri
Concentration of OTA recovered from spiked samples (μg/ml)	9.3	9.2	8.6	8.7	9.5	6.7
	9.1	8.8	9.4	8.4	9.3	6.4
	9.7	9.5	9.8	9.3	9.2	8.9
Recovery % (Mean ± standard deviation)	93.6 ± 0.31	91.7 ± 0.35	92.7 ± 0.611	88.0 ± 0.46	93.3 ± 0.15	73.7 ± 1.33

2007) for fungal growth and toxin synthesis (FAO, 1983, p. 103) accounts for the high susceptibility of the grain to the toxin.

Millet and sorghum are two African traditional crops that were replaced by maize as main staples in the last three decades because the former has higher yield. For this reason, and the fact that they are not export crops, the grains particularly millet have been neglected leading to very limited reports on mycotoxins in them in Africa. The report of JECFA (2008) on OTA contamination in cereals including millet from Nigeria, Ghana and Burkina indicates very low prevalence of the toxin in Nigerian millet at concentration below 5 μg/kg. Sangare-Tigori et al. (2006) found OTA at toxicologically significant levels of between 17 and 204 μg/kg in millet from Côte d'Ivoire. The absolute presence of OTA at unsafe levels (>5 μg/kg) in all samples analyzed in this investigation which is in agreement with values obtained in the Ivoirian study is one of the very few reports of OTA contamination in millet grown in Nigeria and indeed West Africa. However, the toxin's presence in millet and millet based feeds in other part of the globe is well documented (Dawlatana et al., 2002; Duarte et al., 2010; European Commission, 2002; Thirumala-Devi, Mayo, Reddy, & Reddy, 2002).

The volume of data on OTA in sorghum worldwide was sufficient enough for *Codex Alimentarius Commission* to initiate development of an additional draft annex for prevention and reduction of the toxin in sorghum to the existing code (CAC/RCP 51–2003) of practice for prevention and reduction of mycotoxin contamination in cereals (CODEX, 2012). This nevertheless is not the case in Nigeria as only two works provide data on OTA in sorghum in the country. Elegbede et al. (1982) found ochratoxin A and B in Northern Nigeria at 53% and 86% prevalence and mean (range) levels of 17.37 (0–52) μg/kg and 32.57 (0–60) μg/kg respectively. The second report by Makun et al. (2009) discovered the mycotoxin in 23 out of 112 mouldy samples analyzed at mean (range) concentrations of 48.86 (0–712) μg/kg. The values obtained in the previous studies were higher than levels observed in the current investigation (mean of 7.72 μg/kg) and is because while Elegbede et al. (1982) studied samples collected mostly during the wet season and were affected by the unusually heavy and protracted rains of the 1976 planting season, Makun et al. (2009) worked with visibly mouldy grains. Such grains that have their moisture content elevated by rains on the field and/or during storage and noticeably mouldy samples usually have abnormally high mycotoxins levels (Hell, Cardwell, Setamou, & Poehling, 2000; Udoh, Cardwell, & Ikotun, 2000).

Regardless of its inherent resistance to ochratoxin contamination because of its natural composition of sesamin, a constituent of sesame oil which inhibits OTA and OTB synthesis by ochratoxin producing *Aspergilli* (Lee et al., 2007), the current work revealed a 100% OTA contamination of sesame seed at very moderate concentrations. Ezekiel, Sulyok, Warth, and Krska (2012) screened 17 sesame seed samples from Plateau State, Nigeria for fungal and bacterial metabolites but did not detect OTA in the samples. Although proven ochratoxigenic *A. ochraceus* were isolated from raw (Jonsyn, 1988) and fermented seeds (Jonsyn, 1990), and OTA detected in fermented sesame seeds (Kpodo & Bankole, 2008), all from Sierra Leone, no report has documented the toxin in the oil seed. This therefore is the first report of OTA contamination in Nigeria and possibly Africa.

Literature search revealed only two investigations on fungi and mycotoxins in acha in Nigeria. Okonkwo and Nwokolo (1978) found low levels of aflatoxins in marketed acha grain samples from Plateau State, Nigeria. Gbodi, Nwude, Aliu, and Ikediobi (1987) working in the same Nigerian State, screened acha grain for fungi and seven mycotoxins including OTA, and detected aflatoxins and zearalenone in the grain but none of the 24 acha samples analyzed via TLC contained OTA. Similarly, Ezekiel, Sulyok, et al. (2012) using LC/MS/MS detected 48 fungal and 4 bacterial metabolites in 16 samples of acha from Plateau State but none contained OTA. The herein presented results show the prevalence of ochratoxin A in acha to a maximum level of 22.40 μg/kg. This is the first report of ochratoxin A contamination of acha.

Cassava does not seem to be prone to mycotoxin contamination as the very prevalent tropical aflatoxins were not found in the chips from Benin (Gnonlonfin, Hell, Fandohan, & Siame, 2008), Tanzania (Muzanila, Brennan, and King (2000)), Nigeria (Jimoh & Kolapo, 2008) and Ivory Coast (Kastner et al., 2010). But with unwholesome processing practices, storage under poor facilities and marketing of high moisture products, aflatoxin (Essono et al., 2009) and other mycotoxins (Wareing, Westby, Gibbs, Allotey, & Halm, 2001) become prevalent in cassava chips and products. This could be the case of OTA contamination of the cassava and cassava products. Although there is no report on OTA in cassava chips, the toxin was recovered in an exceptionally rare investigation in 12 out of 19 mould-covered inocula for attie'ke' production and 4 of 5 attie'ke' (a traditional fermented Ivorian cassava product) samples at levels of up to 0.2 μg/kg by Kastner et al. (2010). There are quite a few reports of aflatoxins in garri (Ibeh, Uraih, & Ogonor 1991; Opadokun, 1992, p. 50) but the present study seems to have generated the first data on OTA in garri in Nigeria and possibly Africa. Njobeh, Dutton, Chuturgoon, et al. (2010) screened for OTA but did not find it in Cameroonian garri.

Table 2
Ochratoxin A levels (μg/kg) in some marketed cereals, oilseed and cassava product in Niger state, Nigeria.

Foodstuff	Maize	Millet	Sorghum	Sesame	Fonio(Acha)	Garri
Total samples	17	18	17	19	20	18
Contaminated samples	16	18	16	19	20	18
OTA contamination %	94.1	100	94.1	100	100	100
Range (μg/kg)	0–139.2	10.20–46.57	0–29.50	1.90–15.66	1.38–23.90	3.28–22.73
Mean ± SD (μg/kg)	26.96 ± 35.39^{abcd}	24.74 ± 6.52^{efgh}	8.28 ± 6.23^{ae}	8.14 ± 3.23^{bf}	6.71 ± 5.26^{cg}	7.63 ± 4.07^{dh}
Number of samples with OTA exceeding EU limits	15	18	12	13	13	10

Note: Concentrations are presented as mean ± standard deviation and range in μg/kg. Mean values with similar superscripts are significantly different at 95% confidence level.

This study showed that virtually all (107 out of 109 analyzed) the six major Nigerian food and feed commodities tested were contaminated by the nephrotoxic OTA at levels regarded as unsafe by the EU limit of 5 μg/kg (CEC, 2006). Eighty one of the 109 tested samples had OTA concentrations above 5 μg/kg. Such high incidence of mycotoxin in raw and processed agricultural products even in natural mycotoxins insusceptible crops (sesame, cassava products) is as a result of many compelling factors. Chiefly amongst the factors is the warm (average annual temperature of 31.7 °C) and humid (average annual humidity of 51.6%) climate of Niger State that is favourable for proliferation of *A. ochraceus* and OTA production (Duarte et al. (2010)). According to the traders, the grains were harvested and dried on flat concrete ground and stored in sacks under ambient conditions for over six months. The commodities were sold in ventilated containers. Unwholesome trade practice of mixing mouldy grains of low grade with high quality products in order to maximize profits is common. Non enforcement of regulatory limits on locally grown crops sold in informal markets and general ignorance of the existence of mycotoxins by traders who donated or sold the studied samples was also noted. These factors could account for the aggravated mycotoxin burden of the region (Wagacha & Muthomi, 2008). Foods and feeds contamination with OTA at frequency and levels observed in this investigation could impact negatively on human (Reddy & Bhoola, 2010) and animal health particularly the susceptible pigs and poultry animals with resultant economic losses caused by reduction of production performances (Battacone, Nudda, & Pulina, 2010). The long retention time of OTA in serum of pigs and other animals and therefore the persistent carryover of the toxin into edible animal tissues and products) (Duarte, Lino & Pena, 2011) and its attendant public health impact might be exacerbated in such area with high OTA prevalence. This is particularly so in the study area as animals are fed with visibly mouldy feedstuffs. It might therefore be useful to add recommendations on appropriate good agricultural and manufacturing practices, public enlightenment of farmers and traders on mycotoxins, and enforcement of regulatory limits on both local and imported products in order to reduce the hazards of mycotoxins.

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