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# Natural occurrence of ochratoxin A in some marketed Nigerian foods

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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.

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## 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

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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

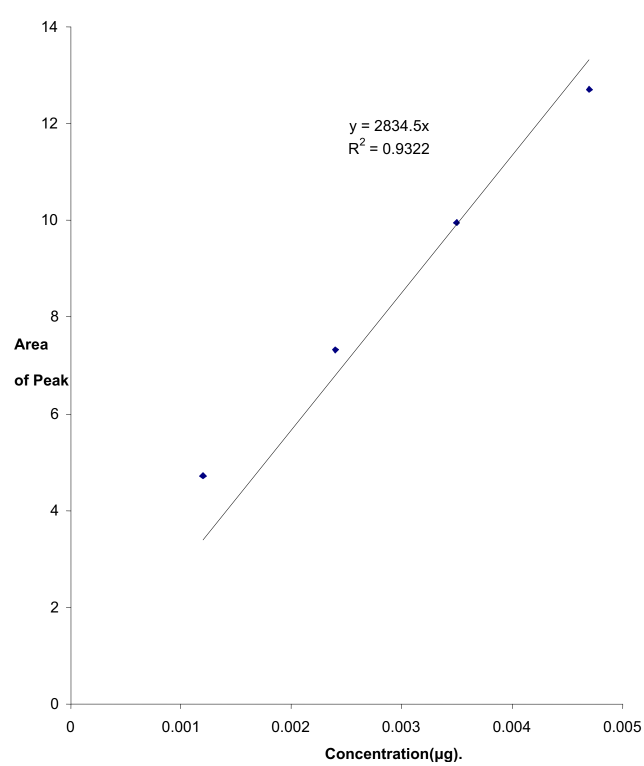


**Fig. 1.** Calibration curve for ochratoxin A standard.

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