©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2550-794X
IDOSR JOURNAL OF SCIENTIFIC RESEARCH 3(2) 1-13, 2018.

Fungal contaminants and aflatoxin content of *Vigna subterranea* (bambara groundnut) flour sold in Nsukka, Nigeria.

*Aroh, K.E.

Department of Microbiology, University of Nigeria, Nsukka.

ABSTRACT

Bambara groundnut (okpa) flour is used to make different kinds of food delicacies cherished and consumed by a large number of people in virtually every part of the world and particularly in Nigeria. However, seed quality, processing and handling methods have not been standardized resulting in products with varying quality and safety levels due largely to contamination by mycotoxins, hence, the need to determine the aflatoxin content of these food products. To isolate and identify fungal-contaminants, investigate contamination level, presence, prevalence and distribution of Aflatoxins in open market bambara flour based on factors such as the state of the bambara nut used in making the flour, duration of flour storage with the aim of developing useful processing and handling practices for acceptable public health standard**.** A total of 32 samples comprising 5 samples each from 6 selected open markets were randomly collected using sterile polythene bags and 2 samples (control) prepared by the researchers from whole and weathered bambara seeds. These were analyzed for microbial quality and aflatoxins content using standard procedures. Five fungi species, *Aspergillus flavus, A. versicolor, A. niger*, Penicillium sp. and Mucor sp. were detected and isolated. Mucor sp. and *A. flavus* had joint highest mean per centage distribution (98.3%) amongst the samples analyzed. Aflatoxins B_1, B_2, G_1 and G_2 were detected in varying concentrations in the various open market samples analyzed with an average occurrence rate of 16.7%. Sampled market bambara flour was found to contain high fungal load and aflatoxins. The nature of the seed used in preparing the flour, hygiene level, duration and exposure of the flour, were the most important factors found to be associated with aflatoxin contamination level of market bambara flour. Results are useful in developing and establishing public health standards for the safe processing, distribution and storage duration of bambara flour.

Keywords: Bambara groundnut, Fungal contaminants, Nsukka, Nigeria.

INTRODUCTION

Bambara groundnut (BG) flour is a food product of bambara groundnut (*Vigna subterranea*) seed prepared by first dehulling the dried seed and the cotyledons winnowed-off, then, milled into flour and sieved to get a fine powdery product, which is the flour. The flour makes a complete food (containing sufficient quantities of protein, carbohydrate, and fat) and is an important source of food security, because it is highly nutritious food for man and animals [1]; [2]; [3]. The flour has the potential to provide a balanced diet in areas where animal protein is scarce and/or expensive [2]; [1].

The bambara seeds are used to prepare different kinds of food, some of which are very important part of human diet. Fresh harvested BG seed can be eaten boiled, or dried seed fried/roasted and eaten as snacks with palm kernel or dried seed milled into flour and used to prepare different kinds of delicacy. In Nigeria, BG

is mostly grown in the north and consumed in the south, especially in the south east.

The processed and cooked BG flour popularly called 'okpa' in the south east, Nigeria, is the most popular form in which BG product is consumed in the West African sub-region. It is consumed by millions of people regardless of ethnicity, religion and socio-economic class making it one of the commonest meals amongst the rich and the poor in Nigeria. Generally, 'okpa' is relished by virtually every Nigerian, and consequently, receives high patronage from among people of all ages, sex, and socio-economic status. 'Okpa' receives high patronage particularly from travelers, students, and families. Among students of high institutions, 'okpa' is popularly called "six-to-six" (that is, 6am to 6pm food) because when eaten as breakfast it fills the stomach and reduces hunger for a long time. Most families in Nigeria, especially average families use 'okpa' as their breakfast usually eaten alone, with pap or rice depending on choice. According to [5], 'okpa' is a better diet for the diabetics than moin-moin, as they were found to have lower values of glycemic index and blood glucose response when 'okpa' was administered; and this may be attributed to the higher crude fibre content of BG than cowpea (moin-moin) [6].

The high consumption of BG foods explains the increase in world production of BG from 29, 660tonnes in 1961 to 79, 160tonnes in 2008 [2]. From the foregoing, it is evident that BG is known and consumed in virtually all parts of the world. The nut is grown across the world mainly for its edible and nutritionally rich seeds. In Africa, BG is the third most commonly eaten legume after groundnut and cowpea [5].

Often, the harvested mature seeds are sun-dried and stored in warehouses or granaries while others are hauled to the markets where they are heaped in hundreds or thousands for sale. In modern age, food packaging and handling have become very important because of the protection of the seeds and its products from contamination by macro- and micro-organisms, prevention from gain of moisture, and shielding the product ('okpa' flour) from air exposure and other sources of contamination [6]. Bambara groundnut contamination may occur pre-harvest (in the farm) or post-harvest (after harvest) [6]; [7]; [8]. Most of the seed infestation and microbial contamination occur post-harvest of the seed due largely to poor handling, storage, and distribution. In the topics, Nigeria inclusive, where the seeds are mostly grown and consumed, the humid and warm tropical conditions often affect the seeds and predispose them to insects (mainly mites, weevils) and other pests such as rodent infestation and consequent contamination by various groups of microorganisms, especially moulds [9]; [10]; [11]. Poorly dried seeds are more susceptible to insect infestation and microbial contamination especially moulds [12]. Weathered seeds are also known to be more prone to microbial contamination.

Whole BG seed milled into flour can also get contaminated during and after processing. The flour is often contaminated due to poor handling, storage, and distribution. In the open markets, the flour is usually displayed in open basins, bowls, jute bags, and sometimes, on mats, polythene bags, and bare tables where the flour gets contaminated by dusts that contains spores of many fungi, particularly during the dry season. The flour can also get contaminated by the cups and bowls used in measuring the flour, which are rarely washed or cleaned year-to-year. Fungal, especially moulds contamination of the seed or the flour could progressively result in fungal proliferation, and species if toxigenic may produce mycotoxins. Studies have shown that among all the mycotoxins studied, aflatoxins are the most lethal. Aflatoxin-contaminated foodstuff poses a serious health hazard to the unsuspecting consumer [13], especially in Africa, Nigeria inclusive, where aflatoxin exposure is more likely to occur due largely to environmental conditions, poor methods of food

handling, poor storage facilities, poverty leading to hunger and poor nutrition, and lack of efficient food monitoring and regulations. In a 2013 awareness workshop organized by the National Agency for Food, Drug Administration and Control (NAFDAC) in collaboration with the International Institute of Tropical Agriculture (IITA), it was noted that some of the agricultural produce in Nigeria earmarked by European Union (EU) for prohibition for export due to high-level of mycotoxin, especially aflatoxin contamination include lemon seeds, cocoa beans among others [14]. One can imagine how contaminated are the poorly-screened or non-screened BG flour sold in our local markets and which we consume almost on daily basis.

Mycotoxin exposure is almost accidental and difficult to avoid. The estimate usually given is that one quarter (25%) of the world's food crops are contaminated with variable levels of mycotoxins [15]; [16]; [17], and people are consuming these toxins in ignorance, compromising their health. This has caused serious economic losses and health problems for individual crops, livestock and poultry farmers, grain handlers, food processors, food vendors, and above all, consumers. Consumption of mycotoxin, especially aflatoxin contaminated foods, milk or meats have been linked to chronic diseases such as liver cancer, kidney diseases, neurological problems, poor development in children, and many other diseases [18];[19]; [16], [19]; [20]; [21]. In some cases, consumption of high levels of aflatoxins has resulted in deaths of animals and human beings. In Kenya (in the eastern and central provinces), for instance, consumption of maize contaminated with aflatoxins resulted in about 125 to 200 deaths in 2004 [21]. It is believed that a 1974 Indian outbreak of hepatitis in which 100 people died may have been due to the consumption of maize that was heavily contaminated with aflatoxin [22]. In 2001, there was also a report in South Africa of serious development of illness in school children fed with aflatoxin-contaminated peanut-butter used in preparing their sandwich meal [23]. Studies in Nigeria also show high levels of mycotoxins, particularly aflatoxin contamination of maize, garri, kwili-kwili and groundnut. Although the total number of people affected by mycotoxicoses is believed to be smaller than the number afflicted with bacterial, protozoan, and viral infections, fungal diseases are nevertheless a serious international health problem.

Although aflatoxin and most Fusarium-mycotoxin can be reduced by cooking the food beyond the normal cooking temperature, studies show they are heat-resistant, thus are stable or moderately stable in most food processes from where they enter the human tissues through food ingestion. As a result, aflatoxin-contaminated food whether eaten raw or cooked still pose a serious health hazard to the unsuspecting consumer. The concern now becomes, how safe are the BG flour sold in our various local markets? This question, consequently, reinforce the need for this research and the need for continuous and regular search for aflatoxin-producing fungi in BG flour, as well as the level of aflatoxin contamination with the aim of ensuring that the BG flour sold in our local markets are safe for human consumption and to save the public from the agony of ill-health and avoidable death.

Materials and Methods

Source of samples and sampling: The samples used for this study were BG (okpa) flour. The flour were grouped into 3 categories – the open markets flour, freshly ground bambara groundnut (BG) whole seed flour, and weathered BG seed flour. The open markets flour (group I) was obtained from 6 selected markets (Eke-Ozi, Obollo-Afor, Ogige, Orie Igbo-Eze, and Obollo-Eke) within Nsukka, Enugu State, Nigeria. A total of 5 samples each were randomly collected from the selected markets, thus, a grand total of 30 samples were collected from the open markets. The group II sample was bambara nut flour processed from a weathered and weevil infested bambara seeds. The group III sample was bambara

flour processed from whole bambara seed. The group III sample was analyzed at 1 week intervals for 1 month starting from the first day the flour was prepared, and was maintained at room temperature. The market samples (approximately 500g/pack) used for the experiment were collected in a new and clean polyethylene pack by adopting standard procedures for collection before transporting to the mycology laboratory of University of Nigeria, Nsukka, for analysis. These samples were analyzed within 24 hours of collection.

Sample Analyses

Isolation Procedure: Ten grams (10g) proportion of each sample was aseptically taken (after thorough mixing) and weighed into a clean and sterile Erlenmeyer's flask containing 100ml of 0.1% sterile peptone water (w/v) and allowed to soak for 2-5 minutes with occasional stirring with a sterile glass rod. Ten-fold serial dilution was subsequently prepared by using sterile micropipette to transfer 1ml aliquot into 9ml of sterile peptone water as diluents. Thereafter, 1ml of each dilution was aseptically plated on potato dextrose agar (PDA) supplemented with chloramphenicol at a concentration of 0.05mg/ml of PDA to kill the bacterial contaminants in the sample using pour plate technique, and then incubated at room temperature for 2-3days. The media used were prepared and incubated according to the manufacturer's instructions. After that, the total viable counts of various colonies were studied and recorded for each of the dilution. The representative colonies (growth) were picked using a sterilized inoculating loop and aseptically subcultured on a fresh sterile PDA and incubated for 2-3days to obtain pure cultures. The cultural and morphological characteristics of the fungal isolates in terms of colour, texture, and pigmentation on the reverse of the plates were recorded. At this stage, all the fungal isolates were presumptively identified based on their cultural and morphological characteristics. The pure cultures obtained were separately grown on PDA block as described by [24]. The fungal isolates were identified based on examination of the characteristic spore structures, sporangiophore or conidiophores structure, and mycelial characteristics using standard mycology manual by [25] and [26].

Aflatoxin Extraction and Detection

50g of the various bambara ground flour samples was homogenized and weighed into 500ml-volume sterile, clean Erlenmeyer flask containing 250ml acetone-water (85+15 v/v), the flask securely stoppered and shaken on a mechanical shaker (Griffin and George, England) for 45 minutes. The resultant slurry was filtered through Whatman filter paper number 4 and clear filtrate collected in 250ml graduated cylinder. Filtrate showing more than a slight opalescence was refiltered. 150ml aliquot of the clear filtrate was taken and transferred to 400ml beaker. To a 600ml beaker, 170ml of 0.02N Sodium hydroxide and 30ml Ferric chloride slurry was added and mixed well. To the filtrate in 400ml beaker was added 3g basic copper carbonate and mixed well. The mixture was then added to the mixture in 600ml beaker. To this mixture was added 150ml diatomaceous earth and mixed well. The mixture was then filtered using Buchner funnel using Whatman filter paper number 4. From the filtrate, 150ml filtrate was quantitatively transferred to 500ml separator, and 150ml 0.03% sulphuric acid and 10ml chloroform added. The mixture shaken vigorously for about 2 minutes and allowed to separate. Then, 13-14ml lower layer of chloroform transferred to 125ml separator. To the separator, 100ml of potassium hydroxide wash solution was added and swirled gently so as not to create emulsion that could be difficult to break, and allowed to stand for 30 seconds for phase to separate. 3ml chloroform layer was collected in 10ml glass stoppered cylinder for chromatography. Emulsions that formed in some cases was drained into

10ml glass stoppered flask and 1g anhydrous sodium sulphate added to it, stoppered and shaken for 30 seconds and allowed to phase separate (chloroform phase need not be completely clear). Emulsions that did not break with this treatment was again transferred to 125ml separator and washed with 50ml 0.003% H_2SO_4 . 3ml chloroform layer was collected in 10ml glass stoppered cylinder for chromatography. 2ml of chloroform solution was transferred to mini-column using 5ml syringe with 5inches, 15 gauge needle. The mini-column was allowed to drain by gravity for about 15-30minutes for column development. When solvent reached top of adsorbent, 3ml elution solvent (chloroform – acetone (9+1)) was added. The column was allowed to drain by gravity until solvent again reached top of adsorbent. Care was taken to prevent the column from running dry during the washing steps. Developed mini-columns were viewed in a UV light cabinet (darkened room used) at wavelength of 365nm. The presence of aflatoxins was indicated by a narrow blue fluorescent band at the top of florisil layer (ca 2.5cm from bottom of column). Some degree of quantification was by comparison with columns developed in extracts with known aflatoxin contents i.e. total aflatoxin levels of the samples were assessed by reference to the control mini-column spiked with known amounts of aflatoxin. Approximations of their total aflatoxin levels were obtained by repeating the procedure using an appropriately smaller aliquot of extract and re-assessing against the control (Makor Chemicals Ltd., Jerusalem, Israel).

Data Analysis

The various data obtained from the laboratory tests were subjected to one-way analysis of variance (ANOVA) and Pearson correlation test. Significance differences were determined at $p<0.05$. The ANOVA results were expressed as mean and standard deviation (SD). This analysis was estimated using computer software known as Statistical Package for Social Sciences (SPSS), version 18.

Results

Results of the investigation of the fungal load and aflatoxins content of market bambara flour sold for human consumption from 6 selected markets in Nsukka, Nigeria are shown in table 1- 6. Five fungi species detected and isolated were *Aspergillus niger, A. versicolor, A. flavus,* Penicillium sp., and Mucor sp. (table 1). Mucor sp. and *A. flavus* had joint highest mean per centage distribution (98.3%) amongst the samples analyzed followed by *A.niger* (91.0%), Penicillium sp. (61.7%), and *A. versicolor* (31.7%) (table 2). Colonidophores forming units (cfu/g) of viable fungi were counted and recorded in all the market samples analyzed (table 3). Slight variations were observed amongst the groups of fungi within each market and from one market to another, and were significantly different at ($p<0.05$). Colonidophores forming units (cfu/g) of viable fungi were also counted and recorded in all the whole bambara nut flour samples (control) analyzes in 4 weeks (table 4).

Table 5, shows the range and per centage occurrence of total aflatoxins in market bambara nut flour samples analyzed. Aflatoxins range were in the order: Obollo-Afor (7.256 – 17.255µg/kg) > Orie Igbo-Eze (8.255 – 16.250 µg/kg) > Ogbede (7.256 – 14.252 µg/kg) > Ibagwa–Aka (5.750 – 12.516 µg/kg) > Eke – Ozi (6.756 – 12.326 µg/kg) > Ogige – Nsukka (8.255 – 10.752 µg/kg). While the rate of occurrence were in the order: Orie Igbo – Eze (21.2%) > Ogbede (16.4%) > Ibagwa – Aka (16.3%) > Ogige – Nsukka (16.2%) > Obollo – Afor (15.3%) > Eke – Ozi (14.7%). Aflatoxins B_1, B_2, G_1, G_2 were variously detected and quantified from all the market samples analyzed. Nevertheless, slight variations were observed in the mean total aflatoxins from one market to another. Table 6, shows the total aflatoxin content and aflatoxin production index of the whole-nut flour samples (control) carried out within 1month at weekly intervals. The results showed slight increase in aflatoxin contamination level with increase in

storage time. The aflatoxin production index (API) range was 2.53 – 11.48 µg/kg/day. The results showed correlation at $p<0.05$.

Table 1: Different fungi isolated from different sources.

Open market bambara nut flour	Weathered seed bambara nut flour	Whole seed bambara nut flour
A. flavus	*A. flavus*	**Week 1:** Mucor sp and
A. versicolor	*A. versicolor*	*A. niger*
A. niger	*A. niger*	**Week 2:** Mucor sp,
Penicillium sp	Penicillium sp	*A. niger*, and
Mucor sp	Mucor sp	*A. flavus*
		Week 3: Mucor sp,
		A. niger,
		A. flavus,
		Penicillium sp and
		A. versicolor
		Week 4: Mucor sp,
		A. niger,
		A. flavus,
		Penicillium sp and
		A. versicolor

Table. 2: Mean per centage distribution of fungal isolates in market bambara nut flour samples.

Market	Types of fungi (%)				
	A. niger	*A. versicolor*	*A. flavus*	*Penicillium sp*	Mucor sp.
Obollo-Afor	80	40	90	80	90
Ibagwa-Aka	100	20	100	40	100
Orie Igbo-Eze	80	50	100	70	100
Ogige- Nsukka	100	20	100	70	100
Eke-Ozi	)0	30	100	60	100
Ogbede	100	30	100	50	100
Mean %	91.7	31.7	98.3	61.7	98.3

Table 3: Fungal load (cfu/g) of market bambara nut flour samples

Sample	Obollo-Afor	Orie Igbo-Eze	Ibagwa-Aka	Ogige-Nsukka	Eke-Ozi	Ogbede
1	8.2×10^4	7.5×10^4	4.8×10^4	5.1×10^4	5.5×10^4	3.8×10^4
2	5.6×10^4	6.8×10^4	3.2×10^4	3.9×10^4	4.8×10^4	5.2×10^4
3	5.4×10^4	5.8×10^4	5×10^4	4.3×10^4	3.1×10^4	4.8×10^4
4	3.4×10^4	4.2×10^4	5.7×10^4	5×10^4	3.4×10^4	6.4×10^4
5	4×10^4	5.4×10^4	4.2×10^4	4.9×10^4	3.3×10^4	3.3×10^4

Table 4: Fungal load of whole bambara nut flour samples (control) at 4 weeks duration

Week	Cfu/g	aflatoxin content (µg/kg)
1	3.2×10^3	3.218
2	3.4×10^3	3.922
3	3.7×10^3	5.443
4	4×10^3	5.628

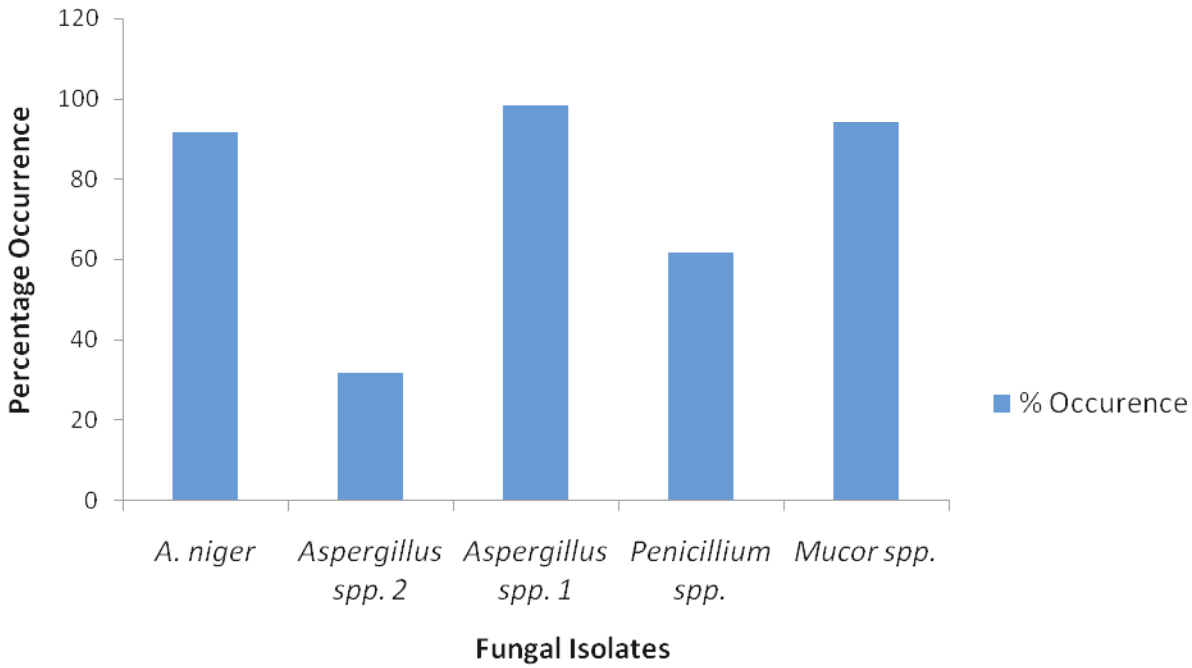
Table 5: Range and per centage occurrence of total aflatoxin in market bambara nut flour.

Market	No. of samples	Total aflatoxin range (µg/kg)	Mean total aflatoxin (µg/kg)	Per centage occurrence	Aflatoxin(s)
Obollo-Afor	5	7.256 – 17.255	9.10	15.3	B_1, B_2,G_2
Ibagwa-Aka	5	5.750 – 12.516	9.699	16.3	B_1, B_2, G_1
Orie Igbo-Eze	5	8.255 – 16.250	12.596	21.2	B_1,B_2,G_1,G_2
Ogige-Nsukka	5	8.225 – 10.752	9.679	16.2	B_1, B_2, G_1
Eke-Ozi	5	6.756 – 12.326	8.733	14.7	B_1, B_2, G_2
Ogbede	5	7.255 – 14.252	9.780	16.4	B_1, B_2, G_1,G_2

Table 6: Total aflatoxin content and aflatoxin production index of whole nut bambara flour at weekly intervals.

Week	Total aflatoxin (µg/kg)	Aflatoxin production index (µg/kg/day)
1	3.218	2.53
2	3.922	3.35
3	5.443	10.60
4	5.628	11.48

Figure 1: Comparison of the mean percentage occurrence of the fungal isolates from the market bambara groundnut flour samples



DISCUSSION

The present study showed that out of 30 market bambara flour sampled, all had detectable level of aflatoxins to Romer mini-column which indicated evidence of aflatoxin contamination and revealed the high presence of aflatoxins in market bambara flours in the area. Aflatoxins detected may be as a result of combination of factors which may include the use of weathered bambara seeds in making the flour, the level of hygiene of the milling machine, inadequate post-processing handling practices such as sieving the flour with unclean and contaminated mesh, measurement of the flour with unclean bare-hand and contaminated cups or bowls, open display of the flour in basin and bowls to the messy of these ubiquitous moulds, prolonged storage of the flour, and non-microbiologically determined hessian bags for packaging and haulage. Others may be the ability of these moulds to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of bambara flour. Previous reports support these findings, [28] and [29]. Based on the result of this work, all the market samples tested positive to aflatoxins with the following range of total aflatoxin contamination level: Obollo-Afor (7.256 – 17.255µg/kg) > Orie Igbo-Eze (8.255 – 16.250 µg/kg) > Ogbede (7.256 – 14.252 µg/kg) > Ibagwa – Aka (5.750 – 12.516 µg/kg) > Eke – Ozi (6.756 – 12.326 µg/kg) > Ogige – Nsukka (8.255 – 10.752 µg/kg). Similar studies in Nigeria on garri showed variable aflatoxins content in garri in Southern Nigeria. Ogiehor et al. (2007) studies showed total aflatoxin range in Anambra (0.44-3.69 µg/kg), Enugu (0.37-5.71 µg/kg), Cross River (0.32-4.57 µg/kg), Edo (0.13-4.46 µg/kg), Delta (0.26-3.64 µg/kg), Imo (0.14-3.16 µg/kg), Rivers (0.17-4.14 µg/kg), Lagos (0.012-2.54 µg/kg), Ondo (0.18-2.41 µg/kg), and Ogun (0.25-1.66 µg/kg). These results were significantly lower than the total aflatoxin content observed in this study. However, some results of this study were within and above the international permissible level, and pose a source of concern on the safety level of bambara flour on display in our local markets. For instance, the EU sets limits for AFB_1 and for total AFs (B_1, B_2, G_1, G_2) in foods (nuts, dried fruits, cereals and spices). Limits vary according to the commodity, but range from 2-12µg/kg for AFB_1 and from 4-15µg/kg [30] in more than 75 countries around the world whilst they are 2-4µg/kg in the European Union (EU) [15]. The maximum residue levels for total AFs and also for the most toxic of them (AFB_1) according to the EU Commission Regulations are 2 and 4µg/kg, respectively. The maximum legal limit for AFM_1 in milk and milk products is set at 0.050µg/kg (50ppt) for EU Member States [15]. Limits of 0.10µg/kg for AFB_1 and 0.025µg/kg (25ppt) for AFM_1 have been set for infant foods [15]. The European Committee Regulations (ECR) has established the maximum acceptable level of AFB_1 in cereals, peanuts and dried fruits for direct consumption in 4ng/g for total aflatoxins (AFB_1, AFG_1, AFB_2, AFG_2) and 2ng/g for AFB_1 alone (Ricci et al., 2007). In USA, the U.S. Department of Agriculture and the U.S. Food and Drug Administration (FDA) have established an "actionable" level of 15-20 ppb or 20µg/kg for foods except milk and a limit of 0.5µg/kg for AFM_1 in milk [15]. Higher limits apply in animal feed.

The mean total aflatoxins content of the open market samples were in the order: Orie Igbo – Eze (12.596 µg/kg) > Ogbede (9.780 µg/kg) > Ibagwa – Aka (9.679 µg/kg) > Ogige – Nsukka (9.615 µg/kg) > Obollo - Afor (9.105 µg/kg) > Eke – Ozi (8.733 µg/kg), while in the control samples, the total aflatoxin content in the weathered seed flour (control 1) was (14.778µg/kg), the total aflatoxin content in the whole seed flour (control 2) was week 1 (3.218 µg/kg), week 2 (3.922 µg/kg), week 3 (5.443 µg/kg), and week 4 (5.628 µg/kg). In control 2, evaluation of the duration of the flour showed correlation between flour duration and aflatoxin contamination level, and this may be due to the period allowed for degradation of the flour. The result was significant at $p < 0.05$. The wide difference in the total aflatoxin content in

open market samples and the whole seed flour samples (control 2) could be as a result of the duration of the flour storage before being sold by the flour vendors which allowed for the flour degradation and fungal growth and proliferation, poor hygiene level during preparation of the flour and exposure to fungal spores in the air in the open markets. Similarly, the relative close values of aflatoxin content in open market flour samples and weathered seed flour samples (control 1) could be as a result of the use of unhealthy bambara seeds in preparing the open market flour. [5] reported that aflatoxins presence in foods is enhanced by factors as stress or damage to the crop due to drought before harvest, insect activity, soil type and inadequate storage conditions. The mean differences in the aflatoxin content of all the open market samples were significant at $p<0.05$.

The high rate of occurrence and prevalence of aflatoxins (total aflatoxins) observed and recorded from all the open market samples investigated may be related to the high rate of occurrence and distribution of moulds such as *Aspergillus niger* (91.7%), *A. flavus* (98.3%), *A. versicolor* (31.7%) and *Penicillium* (61.7%). These group of moulds have been previously linked with the production of various types of mycotoxins under various conditions and supports previous findings [27]; [30]; [29].

Several studies have shown that exposures to aflatoxins and through ingestion of contaminated foods and inhalation of toxins have been linked to acute and chronic toxicity in animals. Effects such as acute liver damage, liver cirrhosis, induction of tumours and teratogenic and other genetic effects in animals and humans are well documented [28]; [20]; [31]. Furthermore, since bambara flour require processing before consumption, there is the possibility of the flour getting highly contaminated with large dosage of aflatoxins over a period of time with possible health hazards when consumed. Unfortunately, aflatoxin-contaminated food whether eaten raw or cooked still pose a serious health hazard to the unsuspecting consumer Hence, the need to develop good practices in bambara nut seed harvesting and storage, processing, handling and storage of this widely cherished and consumed food to prevent or reduce to the barest minimum aflatoxin contamination.

In conclusion, the present work revealed high fungal contamination of open markets bambara flour and high rate of occurrence and prevalence of aflatoxins in all the market samples investigated. Although, the level of aflatoxins in the flour samples were relatively not too high than the limits set by the EU and U.S., but regarding worldwide regulations and results of many other studies, a considerable number of our samples were contaminated with impermissible high levels of aflatoxins. In our study, the nature of the seed used in preparing the flour and hygiene level, duration and exposure of the flour, were some important factors detected to be associated with aflatoxin contamination of bambara flour.

Due to the important role of aflatoxin contamination, especially negative effects of aflatoxin B1 on public health, efforts should be conducted to prevent the contamination, since prevention is the most economical and practical approach. Proper care before and during seed harvesting, and observation of high-level of hygiene during processing and after processing the flour can help us with achieving this noble goal.

REFERENCES

1. Baryeh, E.A. (2001). Physical properties of bambara groundnuts. Journal of Food Engineering, **47**: 321-326.
2. Belewa, M.A., Fagbemi, T., Dosumu, O.O., and Adeniyi, M.O. (2008). Physico-chemical and anti-nutritional properties of some lesser known tree and leguminous seeds. International Journal Agricultural Resources, **3**(3):237-242.

3. Omoikhoje, S.O. (2008). Assessment of the nutritive value of bambara groundnut as influenced by cooking time. Livest. Res. Rural Dev., **20**(4).
4. Onimawo, I.A., Ijeh, I., Ukoha, U., and Nwachukwu, G.T. (2007). Determination of the glycemic index of steamed cakes using two different legumes – bambara nut (*Vigna subterranea*) and cowpea (*Vigna unguiculata*). African Journal of Biochemistry Research, **1**:142-147.
5. Onimawo, I.A., Momoh, A.H., and Usman, A. (1998). Proximate composition and functional properties of four cultivars of bambara groundnut (*Voandzeia subterranea*). Plant Foods Hum. Nutr. 53, pp 153-158
6. Alcaide-Molina, M., Ruiz-Jimenez, J., Mata-Granados, J., and Luque de Castro, M. (2009). High through-put aflatoxin determination in plant material by automated solid-phase extraction on-line coupled to laser-induced fluorescence screening and determination by liquid chromatography-triple quadruple mass spectrometry. Journal of Chromatography A, **1216**(7):1115-1125.
7. Dienar, U.L., Cole, R..J., Sanders, T.H., Payne, G.A., Lee, L.S., and Klich, M.A. (1997). Epidemiology of aflatoxin formation by *Aspergillus flavus.* Annual Revolution Phytopathology, **25**:249-270.
8.Wilson, D.M., and Payne, G.A. (1994). Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops, pp 309-325. In Eaton, D.L., and Groopman, J.D. The toxicology of aflatoxins. Human health, veterinary and agricultural significance. Academic Press, San Diego, California.
9. Agboola, S.A. (1968). Pattern of crop production in south western, Nigeria. Nigerian Geographical Journal; 31-15.
10. Kayode, J.Y., Lombim, I., and Owonubi, J.J. Crop Science and productions in warm climates. 1st Edition. Macmillan Publishers, London; 1988: 185-188.
11. Ventura, M., Gomez, A., Anaya, I., Diaz, J., Broto, F., Agut, M., and Comellas, L. (2004). Determination of aflatoxins B_1, G_1, B_2 andG_2 in medicinal herbs by liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, **1048**(1):25-29.
12. De Vires, J.W., Trucksess, M.W., and Jackson, L.S. (2002). Mycotoxins and food safety. Kluwer Academic/Plenum Publications, New York, N.Y.
13. Doku, E.V. (1995). Proceedings of the workshop on conservation and improvement of bambara groundnut (*Vigna subterranea*). Harare, Zimbabwe: University of Ghana.
14. Daily Independent, 2013. Accessed from, www.dailyindependentnig.com/2013/11/aflatoxin-major-scare-to-nigerians-agric-fapohunda/
15. FAO. (2007). Food and Agricultural Organization of the United Nations food and nutrition, paper 64. Worldwide regulations for mycotoxins. A compendium. Food and Agricultural Organization, Rome, Italy
16. Fink-Gremmels, J. (1999). Mycotoxins: their implications for human and animal health. Veterinary Questions. **21:**115-120.
17. Mannon, J., and Johnson, E. (1985). Fungi down on the farm. New Sci., **105:** 12-16.
18. Chu, F.S. (1998). Mycotoxins – occurrence and toxic effect, pp 858-868. In Sadler, J., Strain, J., and Caballero, B. Encyclopaedia of human nutrition. Academic Press, New York, N.Y.
19. Hendricks, R..G. (1997). Of sick turkeys, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: research on the influence of aflatoxins on child health in the tropics. Annual

Tropical Medical Parasitology, **91**: 787-793.
20. Lund, B.M., Parker, T.C., and Gould, G.W. (2000). The microbiological, and safety and quality of food. "Toxigenic fungi and mycotoxin" Aspen Inc. Publishers, pp 1490-1517.
21. Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., Decoct, K., and Rubin, C. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ. Health Perspect., Volume 113, pp 1763-1767.
22. Krishnamachari, K.A.V.R., Bhat, R..V., Nagarajan, V., and Tilnak, T.M.G. (1975). Hepatitis due to aflatoxicosis. An outbreak in western India. Lancet, **1**:1061-1063.
23. Okafor, J.I. (2008). Fungal diseases: a serious threat to human existence in recent times. 41st Inaugural lecture, University of Nigeria, Nsukka.
24. Riddell, R.W. (1950). Permanent stained mycological preparations obtained by slide culture. Mycologia, 42:265.
25. Beneke, E.S., and Rogers, A.L. (1969). Medical mycology manual. 2nd edition. Bergers Publishing Company, Minneapolis, Minnesota, USA.
26. Ellis, D., and Kidds, S. (2006). Mycology online. Available at www.mycology.adelaide.edu.au/
27. Beatriz, H.P., and Eliana, B.F. (2000). The occurrence of moulds, yeasts and mycotoxins in pre-cooked pizza dough sold in southern Rio grande de sul. Brazilian Journal of Microbiology, **31** (2): 1-8.
28. Ibe, I.N., Uraih, N., and Ogonor, J.I. (1991). Dietary exposure to aflatoxin in Benin city, Nigeria: a possible public health concern. International Journal of Food Microbiology, **14**:171-174
29. Nkama, I. (1987). Review: prevention, elimination and detoxification of aflatoxins in foods and agriculture. Nigeria Journal of Food Science, **2:**15-21.
30. Ibe, I.N. (1994). Morphological distortion of erythrocytes in aflatoxicosis: any diagnostic value? Journal of Medical Laboratory Sciences, **4**:125-130
31. Uraih, N., and Offonry, S. Inhibition of aflatoxin and their implication in human health. Indian Journal of Medical Research. 1981; **68**: 99-108.