



Aflatoxin Total and Microbial Contamination of Grains, Oil Seeds, Yam Chips and Fish Sold in Maiduguri Market

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Abstract

Aflatoxins are secondary metabolites of fungus *Aspergillus flavus* and closely related species that infects several agricultural commodities. The consumption of contaminated commodities adversely affects the health of humans and animals and also a cause of significant economic losses to producers. This study surveyed the aflatoxin contamination level of cowpea, maize, melon, groundnut, yam chips and fish sold in Maiduguri metropolis. Samples from each product (250 g) were bulked and thoroughly mixed using coning and quartering method to get a representative sample for analysis. Laboratory observations were carried out regarding microbial analysis, proximate composition and aflatoxin content. Samples for aflatoxin detection were classified into three; unsorted samples, sorted samples and sorted/washed dried samples. Fish had the highest bacterial load 2.48×10^6 cfu/ml and *Aspergillus* species were found to be the predominant fungi identified. Maize and groundnut with moisture content of 5.63 and 5.62 had the highest total aflatoxin contamination (320.51 $\mu\text{g/kg}$ and 236.3 $\mu\text{g/kg}$ respectively) both in the unsorted group. Total aflatoxin reduction of (58.82 - 99.99%) was observed in all the sorted samples and sorted/washed dried samples. It can be concluded that commonly sold food in Maiduguri had fungal and Aflatoxin contamination. Food should undergo several rounds of sorting to remove discoloured grains and grains that shows evidence(s) of deterioration to reduce the ingestion of food contaminated with aflatoxin.

Keywords: Aflatoxin, Contamination, *Aspergillus* specie, Bacterial load, Maiduguri, Groundnut

1. Introduction

Evidences abound in literature on the consumption of food infested with mycotoxins in the Sub-Saharan Africa, Nigeria inclusive. In African settings, standards for permissible limit of aflatoxins contamination are often applied to export commodities; most of the locally consumed food and food products are habitually neglected. Staple foods are commonly endangered which often results in health risks in children and immunocompromised population [1, 2].

North-eastern Nigeria is famous for high temperature and humidity, creating favorable conditions for fungal growth [3], especially molds which often produce toxic metabolites [4]. Aflatoxins (AFs) produced by ubiquitous fungus *Aspergillus* species have attracted worldwide attention because they are the most toxic among mycotoxins and potent carcinogens [5, 6]. Globally, several studies have stressed the urgent need of surveying the contamination levels of mycotoxins in baby milk, cereal-based formula, foods, oil seeds and beverages [6]. The variation in drying of yam tubers and the difficulty to maintain low water activity in the yam chips might favours the growth condition of moulds and thus produces AFs [7, 8]. Dried fish is one of the traditionally accepted food in Africa which is a rich source of protein

and a good substrate for fungal growth which may result from improper drying and personal hygiene and thus produces toxic metabolites such as AFs [9].

In Nigeria and other countries, aflatoxin is at a centre stage and considered a serious national problem in food supplies. As a result of the urgent need for research in aflatoxin, we adopt the ELISA method of aflatoxin detection to investigate the menace of aflatoxin infestation of food and food products in the domestic food supply. There is dearth of information on the AFs contamination status of commonly consumed food. In this study we aimed to investigate the contamination level and aflatoxin contents of grains, oil seeds and fish sold in the local market.

2. Materials and Methods

2.1 Sample Collection

A total of thirty samples of Maize, Cowpea, Melon, Groundnut, Yam Chips and Smoked Dried Fish were purchased from Maiduguri market. The samples weighing 250 g each were immediately transferred into a zip lock bag and transported to Nigerian Stored Products Research Institute Kano laboratory for further analysis. The samples from each product were bulked and thoroughly mixed using coning and quartering method to get a representative sample for analysis.

2.2 Sample Processing

Laboratory observations were carried out regarding microbial analysis, proximate composition and aflatoxin contents. Twenty five grams of each of the samples were homogenised and used for the preparation of the stock solution for bacterial load and fungi identification. Samples for aflatoxin detection were classified into three; unsorted samples, sorted samples and sorted/washed dried samples. The samples underwent several rounds of sorting to obtain the sorted samples. Thereafter, samples were obtained from the sorted samples, washed and dried with multi-crop dryer to obtain the sorted/washed dried samples for analysis.

2.3 Bacterial Load and Fungi Identification

The stock solution for each sample were prepared by transferring 1 g of homogenised sample into 9 ml of sterile distilled water. One millilitre of the stock solution was serially diluted up to 10^{-4} in 9 ml of sterile distilled water, and 1 ml of the aliquots were plated on Nutrient agar and Potato dextrose agar, incubated at 37°C for 18-24 h and up to 7 days at room temperature respectively. Colonies were counted manually to determine the bacterial load after 18-24hours of incubation. The fungal growth was identified after 7days of incubation using physical observation and lactophenol cotton blue staining technique.

2.4 Proximate Analysis

Proximate analysis of each sample was carried out to estimate the moisture, crude fiber, ash, protein, oil and carbohydrate contents.

2.4.1 Moisture Content

The moisture content was determined according to AOAC hot-air oven method [10]. Briefly, the empty dish and lid were dried in the oven at 105 °C for 3 h and transferred into a desiccator to cool after which the empty dish and lid were weighed. Three grams of the sample was weighed into the dish and thereafter the dish containing the sample was placed in the oven for 3 h at 105 °C. After drying, the dish was transferred to a desiccator to cool while the lid of the dish partially closed. The moisture content was calculated as follows:

$$\% \text{Moisture Content} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = Weight (g) of sample before drying and W_2 = Weight (g) of samples after drying

2.4.2 Ash Content

The ash content was determined according to the method described by Oko *et al.* [11]. Briefly, a porcelain crucible was fried in an oven at 100 °C for 10 min, cooled in a desiccator and weighed (W_1). Two grams each of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed (W_2). It was first ignited and then transferred into muffle furnace

which was then set at 400 °C. The sample was then left in the muffle furnace for 30 min to ensure proper ashing. The crucible containing the ash was then removed and cooled in the desiccators after which it was weighed (W_3). The percentage ash content was calculated as:

$$\% \text{Ash Content} = \frac{W_3 - W_2}{W_1} \times 100$$

2.4.3 Crude Fat/Oil

The crude fat/oil content was determined using Soxhlet extraction method according to AOAC method [10]. The oil (fat) content of each sample was calculated using the formula:

$$\% \text{ fat + moisture} = \frac{W_3 - W_4}{W_2 - W_1} \times 100$$

Where, W_1 = weight of thimble; W_2 =weight of wet sample + thimble; W_3 = initial weight of sample + thimble + glass wool; W_4 = final weight of sample + thimble + glass wool

2.4.4 Crude Fiber

The crude fiber content was determined according to AOAC method which involves series of washing in acidic and basic solutions [10]. The calculation was done as follows:

$$\% \text{Crude Fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 = weight of sample; W_2 =weight of crucible + sample fiber W_3 = weight of sample + ash fiber

2.4.5 Crude Protein

The crude protein content of the samples was determined as described by Kjeldhal procedure of AOAC [10]. The percentage protein content was calculated using the formula:

$$\text{protein (\%)} = \frac{(A - B) \times 0.05 \times 1.4007 \times 6.25}{W} \times 100$$

Where, A = Titer value of sample (ml), B = titer value of blank (ml), W= weight of sample (0.5 g), Molarity of HCl used = 0.05 M, 1.4007 = equivalent of atomic mass of Nitrogen and 6.25 = conversion factor of nitrogen

2.4.6 Carbohydrate content

The carbohydrate content was determined as the difference between 100% and sum of the percentages of ash, protein, fiber, moisture and oil contents.

Carbohydrate contents were calculated as:

$$\text{Carbohydrate (\%)} = 100 - [\text{protein (\%)} + \text{Moisture (\%)} + \text{Ash (\%)} + \text{Fiber (\%)} + \text{Fat (\%)}]$$

2.7 Total Aflatoxin Determination

The total aflatoxin was determined following the procedure of RIDASCREEN® aflatoxin total which is an

Enzyme Linked Immunosorbent Assay (ELISA). Briefly, 2 g each of the homogenised sample was dissolved in 10 ml of 70% methanol and mixed for 10 min using a shaker. The extracts were filtered using a Whatman No. 1 filter and 100 µl of the filtrate were diluted in 600 µl distilled water for the preparation of the ELISA plate. Fifty microliter of the standards and the prepared samples were dispensed in wells and 50 µl of the conjugate and antibody were added to the well and mixed gently by shaking the plate manually and then incubated for 30 min at room temperature. The content of the wells was poured out by turning the microwell holder upside down vigorously against an adsorbent paper and the wells were washed three times with wash buffers. One hundred microliter of the substrate/chromogen were added to each well and incubated for 15 min at room temperature and finally a stop solution of 100 µl was added to each well. The absorbance was measured at 450 nm within 30 min of adding stop solution using RIDA®SOFT WIN.NET.

2.8 Data Analysis

Experimental analysis was carried out in triplicate and expressed as means ± standard deviation. Data were analyzed using a one-way analysis of variance using SPSS version 20. A p-value of <0.05 was considered indicative of statistically significant differences.

3. Results and Discussion

Colonization of food by bacteria, fungi as well as aflatoxin infestation is an indication of poor handling practices from pre-harvest to post-harvest. Isolation of bacteria and fungi from food samples is an indicator of poor handling of food vis à vis drying and storage techniques. Table 3.1 presents the bacterial count of all the samples studied. Cowpea and fish (2.48×10^6 cfu/ml and 2.04×10^6 cfu/ml, respectively) had the highest bacterial load while melon had the lowest value of 1.1×10^5 cfu/ml. The bacterial load of groundnut was found to be 1.3×10^5 cfu/ml, which is in agreement with the range of values obtained by Oko *et al.* [11] in their analysis of groundnut in Zaria, Nigeria and at variance with Kigigha *et al.* [12] in Bayelsa state Nigeria. The difference may be due to the geographic variation of the sampling sites. Failure to dry to safe moisture levels before bagging and storage as well as spreading grains meant for human consumption on bare floor could be a possible factor for higher bacterial loads recorded. Lower values for the microbial load in melon and groundnut could be attributable to their oily nature which offer extra protection against bacteria colonization.

Fungi are ubiquitous in nature, seemingly all food items are a substrate for their proliferation; they endanger grains, oil seeds and fish leading to deterioration and economic loss to farmers and consumers. *Aspergillus* species were found to be the predominant fungal specie

identified from the samples collected (Table 3.1) which is in accordance with the reports on grains, oil seeds and fish across Nigeria [13,14,15]. Isolation of variety of fungi species may be due to the high temperature and humidity of the sampling location which favours the proliferation of variety of fungi specie [2].

Proximate composition of food samples (Table 3.2) showed that yam chips have the lowest values for all the parameters analyzed except for carbohydrate, which had the highest value (89.88%). Groundnut had the highest ash content (3.02%) which is higher than that reported by Ayoola *et al.* [16] and Alhassan *et al.* [17], but in the range of values (3.08%) reported by Atasi *et al.* [18]. The moisture content of cowpea and fish was found to be the highest (6.94% and 6.71 respectively). Fish samples also had the highest protein content (52.13%). For fats and oils, melon (40.80%) and groundnut (38.26%) were found to exhibit the highest content followed by the fish samples (12.68%).

Infestation of food by fungal species and subsequent contamination with the toxic secondary metabolites (e.g., AFs) is a global challenge posing a threat to the availability of safe food. The aflatoxin contents of the samples are presented in Table 3.3. Total aflatoxin contamination of 236.3 µg/kg and 320.51 µg/kg were observed in the unsorted groundnut and unsorted maize respectively which exceed the acceptable level of European Commission (4 µg/kg) and Food and Drug Administration of the United States with acceptable limit not exceeding 20 µg/kg [19]. The high values obtained in unsorted groundnut and unsorted maize may be due to the handling practices (pre-harvest and postharvest) which favours the proliferation of fungi that produces aflatoxins and a cause for concern for policy makers in Nigeria to enact inspection laws and surveillance of food and food products before consumption by human and production of feeds.

However, a relatively lower total aflatoxin contamination were observed in all sorted, washed and dried samples. This result indicates the need for sorting and washing of grains, oil seeds and fish before consumption. Kumar *et al.* [20] reported 40-80% reduction in AFs levels after sorting. A significant reduction of total aflatoxin contamination was observed in this study ranging from 58.82% to 99.99% after rounds of sorting and washing. Groundnut contamination of 214 ppb was reduced to less than 5 ppb with several rounds of sorting in Haiti [21]. Controlling humidity, temperature and moisture are among the most effective management strategies to combat AFs production during the storage and transport of susceptible commodities.

Table 3.1: Bacterial and Fungal Studies of Samples Collected from Maiduguri Market

Sample	Bacteria count (cfu/ml)	Isolated Organism(s)
Groundnut	1.30 x 10 ⁵	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> ,
Cowpea	2.48 x 10 ⁶	<i>Aspergillus niger</i>
Yam chips	6.40 x 10 ⁵	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>
Melon	1.10 x 10 ⁵	<i>Aspergillus niger</i> , <i>Penicillium specie</i>
Maize	1.88 x 10 ⁶	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Fusarium species</i>
SD Fish	2.04 x 10 ⁶	<i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i>

SD =smoke-dried, cfu = colony forming unit

Table 3.2: Proximate Composition of Samples Collected from Maiduguri Market

Sample	Moisture content (%)	Ash content (%)	Crude fibre (%)	Fat content (%)	Protein (%)	Carbohydrate (%)
Cowpea	6.94±0.10	2.79±0.15	1.90±0.05	1.65±0.03	33.40±1.02	53.32±2.01
Maize	5.63±0.10	1.99±0.07	1.95±0.03	2.00±0.07	6.52±0.50	81.91±1.00
Groundnut	5.62±0.10	3.02±0.09	1.83±0.07	38.26±0.10	25.74±0.95	25.53±0.85
Yam chips	4.15±0.04	1.52±0.10	1.66±0.04	0.86±0.05	1.93±0.01	89.88±2.00
Melon	5.30±0.03	2.30±0.07	4.68±0.02	40.80±0.41	22.54±0.55	24.38±1.02
SD Fish	6.71±0.11	2.03±0.07	3.01±0.09	12.68±0.05	52.13±2.05	23.44±1.05

Values are means ± SD of triplicate experiment. SD =smoke-dried. Carbohydrate (%) = 100 - [protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Fat (%)].

Table 3.3: Aflatoxin Contents of Samples Collected from Maiduguri Market

Sample	Total Aflatoxin (µg/kg)		% AF _{total} Reduction	Washed/dried	% AF _{total} Reduction
	Unsorted (whole)	Sorted			
Groundnut	236.31±6.12	0.021±0.00	99.99±0.00	0.001±0.00	99.99±0.00
Melon	0.119±0.01	0.049±0.00	58.82±0.50	0.003±0.05	97.48±0.01
Maize	320.51±9.27	0.013±0.01	99.99±0.00	0.001±0.00	99.99±0.01
Cowpea	0.002±0.00	ND	ND
Yam chips	0.115±0.75	0.006±0.02	94.78±0.02	ND
Smoked Dried fish	0.591±0.05	0.122±0.00	79.36±0.20	0.037±0.10	93.74±0.01

Values are means ± SD of duplicate experiments. There is significant difference between unsorted, sorted and washed/dried sample (p<0.05). ND = Not Detected.

4. Conclusion

This study showed that staple food consumed in Maiduguri had bacterial and fungal contamination. Fungal contamination of food results in aflatoxin production in the food samples. Rounds of sorting and washing/drying reduces the detection rates of total Aflatoxin in all the samples. It is difficult to identify food contaminated with bacteria, fungi and aflatoxin. Food for human consumption should undergo several rounds of sorting to remove discoloured grains and grains that shows evidence(s) of deterioration to reduce the ingestion of food contaminated with aflatoxin.

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