



## Fungi and Aflatoxin B<sub>1</sub> of Freshly Harvested, Hand and Machine Shelled Groundnuts and Groundnut Cake

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### Abstract

A study was carried out to determine the moisture content, associated fungi and aflatoxin B<sub>1</sub> of freshly harvested, hand shelled, machine shelled groundnut seeds and groundnut cake "Kulikuli". A total of 36 samples, consisting of three 250g samples for each of these were collected from three sellers in Nasarawa Eggon, Lafia, and Agyaragu in Nasarawa state for the study. Moisture content was determined by drying in an oven to constant dry weight at 105 °C for 18 hours. Fungi were isolated by culturing using the blotter method and subculturing onto potato dextrose agar (PDA), and identified based on colonial and morphological characteristics. Results showed that moisture content of fresh groundnut ranged from 32-44% with mean of 36.67%, hand shelled groundnut ranged from 10-12% with mean of 10.67%, and that of machine shelled groundnut ranged from 6-8% with mean of 7.33%. *Aspergillus flavus*, *A. tamarii*, *A. niger*, *Cercospora personata* and *C. Arachidicola* were isolated. At 70% frequency of isolation, *A. flavus* had a significantly ( $P \leq 0.001$ ) higher occurrence than the others. Aflatoxin B<sub>1</sub> was lowest in freshly harvested groundnuts, 19.63 µg/kg and the only one within any existing regulatory limit; and highest in machine shelled groundnut at 338.33 µg/kg. The aflatoxin B<sub>1</sub> content of these basic food and feed commodities should be a concern for health and further studies.

**Key words:** Groundnut, Groundnut cake, Aflatoxin, Mycotoxin, Fungi

### Introduction

Groundnut is second most important legume crop the world over (Okello *et al.*, 2010). It serves as food for humans and livestock; and valuable dietary protein component in the absence of meat (Sim, 2011). It contains 47-53% oil and 25-36% protein (Prasad *et al.*, 2011). Groundnut is used for oil and in feed production. It is also a popular snack in Nigeria as boiled or roasted groundnut and as groundnut cake – Kulikuli (Adebisi *et al.*, 2001; Atanda 2011). Nigeria used to be Africa's highest producer, but production suffered many setbacks including mycotoxin contamination (Ajeibe *et al.*, 2014). Aflatoxins particularly are highly toxic and carcinogenic mycotoxins that occur naturally in agricultural commodities such including maize groundnut (Chala *et al.*, 2013; EFSA, 2017). Aflatoxin B<sub>1</sub> is the most common in food and amongst the most potent genotoxic and carcinogenic aflatoxins (EFSA). Aflatoxin contamination of groundnuts and groundnut products like cake have been documented in several parts of the world and Africa, including Ethiopia, Mali and Ghana (Osiru and Waliyar, 2013; Mohammed *et al.*, 2016) and in some parts of Nigeria (Bankole and Eseigbe, 2004; Afolabi *et al.*, 2015). In Nasarawa state, central Nigeria, information on fungi and aflatoxin contamination of seeds and cake of groundnut are not readily available. This study therefore focused on determining the moisture content of freshly harvested, hand shelled and machine shelled groundnut, the fungi associated these groundnut seeds and groundnut cake, and their Aflatoxin in B<sub>1</sub> content.

## Materials and Methods

### Sampling and moisture content determination

Three 250g samples each of Freshly harvested, hand shelled and machine shelled groundnut seeds and groundnut cake, were collected from three sellers in Lafia, Nasarawa Eggon and Agyaragu in Nasarawa state totaling 36 samples, for the study. Moisture content determined by oven drying to constant dry weight at 105 °C overnight and taking the difference between the initial weight and weight after oven drying.

### Identification of Fungi and Aflatoxin B<sub>1</sub> determination

For each seed type in each location, a 250 g sample was collected from three sellers in the market, a subsample of 10 seeds were plated each per sterile petri dish in three petri dishes to which a Wartman No. 1 filter paper (blotter paper) was placed and moistened with sterile distilled water. The seeds were surface sterilized using sodium hypochlorite 3.5 % for 30 seconds and rinsed in three changes of sterile distilled water. The fungi that germinated from the seeds and groundnut cake cultures of the blotter paper were then transferred to potato dextrose agar - PDA (Oxoid), prepared by dissolving 39g of the anhydrous powder per liter of sterile distilled water, and autoclaved at 121°C at 15 psi. The isolates were further sub-cultured on PDA to obtain pure cultures. Isolates were identified based on colonial appearance and morphological characteristics under the microscope. Aflatoxin B<sub>1</sub> was extracted using Methanol/water + 0.5 KCl (70:30 V/V). Enzyme-linked immunosorbent assay (ELISA) was performed following the ICRISAT protocol (2009) for indirect competitive ELISA assay. The result was read using Biorad imark microplate reader and the optical densities obtained were plotted on a standard graph to determine concentrations.

## Results

The results showed that the mean percent moisture content of freshly harvested groundnut was 38.57 % that of hand shelled groundnut was 10.67 % while that of machine shelled groundnut was 7.33 % (Table 1).

**Table 1: Moisture content of freshly harvested groundnut, hand shelled groundnut and machine shelled groundnut sampled**

Location	Freshly-harvested groundnut (%)	Hand-shelled groundnut (%)	Machine-shelled groundnut (%)
Lafia	40	10	8
Nassarawa Eggon	32	10	6
Agyaragu	44	12	8
Mean	38.67	10.67	7.33

The percentage occurrence of fungi isolated from freshly harvested, hand and machine shelled groundnuts and groundnut cake (Table 2), showed *Aspergillus flavus* ranged from 0.67 % in freshly harvested groundnut and groundnut cake to 70 % in machine shelled groundnuts. The variation in percentage isolations of *Aspergillus flavus* from the various forms of groundnut seeds and cake was highly significant. *Aspergillus tamaris* was not isolated from freshly harvested and hand shelled groundnut, but was isolated from 0.67 % and 1.33 % samples of groundnut cake and machine shelled groundnuts respectively. *Aspergillus niger* was isolated only in freshly harvested groundnuts, and only from 0.67 % samples.

*Cercospora personata* was present in freshly harvested and hand shelled groundnuts at 2 % and 0.67 % respectively. *C. arachidicola* was only isolated in freshly harvested groundnuts and was 0.67 % of isolates.

**Table 2: Percentage occurrence of fungi detected in freshly harvested, hand and machine shelled groundnut seeds and groundnut cake.**

Type	<i>Aspergillus flavus</i>	<i>Aspergillus tamaraii</i>	<i>Aspergillus niger</i>	<i>Cercospora personata</i>	<i>Cercospora arachidicola</i>
†FHG	0.67	-	0.67	2	0.67
HSG	10	-	-	0.67	-
MSG	70	1.33	-	-	-
GNC	0.67	0.67	-	-	-
P value	0.001*	0.567	0.90	0.525	0.452
SD	±27.8	±1.7	±3.1	±1.6	±0.5

FHG =freshly harvested groundnut HSG=hand shelled groundnut MSG=machine shelled groundnut GNC=groundnut cake

*Aspergillus flavus* was also recorded the highest percentage isolations from the three locations sampled (Table 3). It had the highest percentage isolation of 21.6 % in Agyaragu, and the lowest in Nassarawa Eggon (14.8 %). The mean isolation in these locations was 18.26 %. The least isolations were those of *Cercospora personata* and *C. arachidicola* at 0.53 % and 0.13 % respectively.

**Table 3: Fungi associated with the groundnut seeds and groundnut cake in three localities in Nasarawa state.**

Location	<i>Aspergillus flavus</i>	<i>Aspergillus tamaraii</i>	<i>Aspergillus niger</i>	<i>Cercospora personata</i>	<i>Cercospora arachidicola</i>
Lafia	18.4	0.4	24	0	0
Nas./Eggon	14.8	0.4	0	0.4	0
Agyaragu	21.6	1.6	0.4	1.2	0.4
Mean	18.26	0.8	0.93	0.53	0.13

The results of the aflatoxin B<sub>1</sub> assay (Table 4), showed that, the highest mean concentration of the toxin was in machine shelled groundnuts, 338. µg/kg, followed by groundnut cake (Kuli-kuli), at 178. 27 µg/kg and hand shelled groundnuts at 38.76 µg/kg. Freshly harvested groundnut had 19.63 µg/kg.

**Table 4: Aflatoxin B<sub>1</sub> in groundnut and groundnut cake in Nasarawa state (in µg/kg).**

Location	Freshly harvested groundnut	Hand shelled groundnut	Machine shelled groundnut	Groundnut cake
Lafia	17.8	36.4	70.0	53.2
Nasarawa Eggon	33.2	20.5	39.1	239.9
Agyaragu	7.9	59.4	905.1	241.8
Mean	19.63	38.76	338.33	178.27

## Discussion

The moisture content (mc) of freshly harvested groundnut (FHG), 32-44%, agrees with known mc of groundnut at harvest which ranges between 26 -40 % and can be up to 50%. (Durhahm, 2007; Noomhorn and Cardona, 2016). Machine and hand shelled groundnut were 7.3% and 10.67% is below 12-14 % is regarded a safe storage mc that prevents mold growth (Diener

and Davis 2007, Cardona, 2016) prevent mold growth; and within the range of the US grading standard (USDA 2000). Although the moisture content of machine shelled groundnut was within the safe moisture limit, its aflatoxin B<sub>1</sub> content was highest (338.33 µg/kg). It is evident from the *Aspergillus* colonization in table 2 of 70 % of the samples that this may be responsible. It is also comprehensible that machine shelled groundnut seeds, receive more bruising, which will be responsible for the high percentage rate of colonization by the fungus. The bruising could also be responsible for the drying to a low moisture content of 7.3 % as moisture will quickly evaporate from bruises, but this would have come after colonization and the accumulation of aflatoxin would have started and possibly continued to take place. *Aspergillus flavus*, was most occurring fungi in all forms of groundnut and fungal isolates from all location at 18.26%. This agrees with Folagbade (2010) and Chala *et al.*, (2013) that *A. flavus* and *A. niger* were the most prevalent *Aspergillus* species in groundnut. The Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was lower than 20µg/kg US and CODEX limits, only in freshly harvested groundnuts. Nigeria set limit only set for total aflatoxin in groundnut seeds (20µg/kg) but not for Aflatoxin B<sub>1</sub>). The high levels of AFB<sub>1</sub> is attributed to shelling methods as highest was in machine shelled.

It is not surprising that freshly harvested groundnuts contain aflatoxin B<sub>1</sub> as it is known that *Aspergillus flavus* colonization and aflatoxin contamination begins from the field and have been documented (Osiru and Waliyar, 2013). GNC or Kulikuli from interaction with makers uses any seeds shelled by any method, but especially favorite customers of MSG, because groundnut seeds that are visibly poor in appearance are not easily accepted in the market, but for Kuli kuli, since the seeds are not seen, it is easily sold out to the kulikuli processors and accepted by them, this which could be responsible for its high AFB<sub>1</sub> Content, due to high *A. flavus* contamination.

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