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DETERMINATION OF MOISTURE CONTENT, TOTAL AFLATOXIN CONTENT AND MOULD COUNT IN PURCHASED AND PROCESSED GROUNDNUT (*Arachis hypogea* L.) PRODUCTS OBTAINED FROM PARTS OF BENUE STATE-NIGERIA

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Abstract: The evaluation of total aflatoxins (TAF) in purchased and processed groundnut (*Arachis hypogaea* L.) products in some areas (Makurdi and Otukpo) of Benue State, Nigeria was studied using Direct Competitive Enzymes Linked Immunosorbent Assay method (ELISA). Also the mould counts; and mould isolation and identification were carried out done as well as the moisture content. The results of the moisture content indicated that all the collected samples fall within the tolerable limit of 15% for cake, 2% for oil and 30% for paste, respectively. The levels of total aflatoxin in the processed and the purchased samples were within the permissible limit set by NAFDAC (20 µg/kg) and Codex Alimentarius commission (CAC) (10 µg/kg) except Wadata market (oil and paste), Kanshio market paste and North bank market oil in Makurdi area; and Otukpo main market and Ela market pastes in Otukpo area that have TAF marginally above the CAC permissible limit. Furthermore, mold count results showed a total of 203 isolates and Aspergillus flavus with 91 (44.83%) occurrence was observed as the major isolate. In a nutshell, the total aflatoxin contamination analysis revealed no significant differences between the processed and purchased ground nut products. Therefore, is logical that these products were infiltrated by aflatoxin prior to processing and also during their storage. The research has revealed that routine check for aflatoxin in such food products is vital and should not be compromised in order to avoid possible aflatoxin poisoning from the study areas.

Keywords: Aflatoxin, ELISA, food contamination, groundnut

Introduction

Groundnut (*Arachis hypogaea L.*) is a leguminous oilseed crop cultivated in the semi-arid and subtropical regions of the world. About 100 countries cultivate it on six continents between 40° N and S of the equator on nearly 24.6 m ha, with a production of 41.3 m.t. and productivity of 1676 kg ha^{-1} during 2012. Globally, China, India, Nigeria, USA and Myanmar are the major groundnut producing countries. Developing countries in Asia, Africa and South America account for over 95% of total production. However, Americas productivity (3632 kg ha^{-1}) is substantially higher than that of Asia (2217 kg ha^{-1}) and Africa (929 kg ha^{-1}) (Ajeigbe *et al.*, 2014). In addition, literature has it that Nigeria is the largest groundnut producing country in West Africa, accounting for 51% of production in the region. That is Nigeria contributes about 10 and 39% of total production in the world and Africa, respectively. Furthermore, it is also found that as at 2008/2009, Nigeria was the largest producer of groundnut in Africa and 4th in the world (Abdulrahaman *et al.*, 2014). Groundnut has the highest oil content of all food crops and is second only to soybean in term of protein content (20-30%) among the food legumes. Among the many foods we have in Benue State (an extensively agrarian state in Nigeria), groundnut is among the major agricultural products in Benue. Some of the common dishes of the groundnut are the groundnut oil, groundnut cake, groundnut paste etc. These various products of groundnut are heavily consumed (Abdelhamid, 1990). In fact, about 10 products from groundnut have been reported in Nigeria(Abdulrahaman *et al.*, 2014). More so, there gional average daily consumption of groundnut is < 5, 5-10, 10 -15 and 15 – 40g per day for South West, North Central& South East, North West & South South, and North East of Nigeria, respectively (Fapohunda, 2013).

The American Food and Agriculture organization estimated that 25% of food crops are affected by mycotoxins. Of these mycotoxins, aflatoxins are the most important because they cause more severe health issues. Amongst several issues, aflatoxin (especially aflatoxin B1) are potent carcinogen in animals(Ahmed, 2003; Backer *et al.*, 2005; Ajeigbe *et al.*, 2014). Aflatoxins are a group of toxic metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The fungi grow in soil, decaying vegetation, hay, and grains (Eaton and Cropman, 1994). Thus, aflatoxins are regularly found in improperly stored staple commodities such as cassava, chili peppers, corn, cotton seed, millet, groundnut, rice, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices, beans (Diedhiour, 2011). In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) Nigeria has set a maximum permissible level (MPL) of 20 µg/kg for total aflatoxins in all foods for human consumption (Ezekiel *et al.*, 2013; Salau *et al.*, 2017), while the Codex Alimentarius Commission, CAC has MPLs for TAF as 10.0 µg/kg (Ubwa *et al.*, 2014).

Some of the conditions for aflatoxin are; elevated moisture content, improper handling and drying, improper storage conditions, insect infestation or damage (Angelo *et al.*, 2009). In fact, aflatoxin is tolerant under most conditions of storage, handling and processing of food. It is heat stable and withstands temperature up to boiling point of water, thus they even persist in processed foodstuffs (WHO, 2005). Processed foods can also contain aflatoxin (WHO, 2006). In addition, it is noteworthy that when aflatoxin infested food/ feed stock are processed, the aflatoxins enter the general food supply chain as they have been present in both animals and human (Eaton and Cropman, 1994). Animals that are fed on aflatoxin contaminated food can pass it on through products such as; eggs, milk products, and meat. For example, chicken fed with aflatoxin contaminated poultry feed were observed to produced aflatoxin-laden chicken meat and eggs in Pakistan (Saito and Tsuruta, 1993).

As per groundnut, aflatoxin contamination can occur in the field, during postharvest drying and storage, and even during transportation. Crop husbandry practices, mechanical damage, insect and bird damage, climatic conditions (drought, stress or excessive rainfall), and soil factors etc. significantly influence aflatoxin contamination (Angelo *et al.*, 2009; Ajeigbe *et al.*, 2014). Drought during the growing season of groundnut increases the probability of preharvest aflatoxin contamination (due to infection by *Aspergillus flavus*). Aflatoxin contamination of groundnut is a major hazard to human and animal health and is one of the major constraints to the groundnut trade. Risks related to human health and declining productivity in livestock after consuming aflatoxin-contaminated feed have led to groundnut importing countries

setting standards that allow only extremely low levels of contamination and that are often not achievable by most resource-poor groundnut farmers. In addition, delayed harvesting also leads to increase possibility of *Aspergillus flavus* infection, and aflatoxin contamination in pods/seeds (Ajeigbe *et al.*, 2014).

Unfortunately, aflatoxin has been reported to cause severe illness and death in many parts of the world. Aflatoxins have also been reported to reduce immunity in humans and animals. Exposure to aflatoxin also enhances stunting and underweight in children especially those below three years. Aflatoxins have also been implicated in the slowed rate of recovery from protein malnutrition (kwashiorkor). Generally, from the animal health perspective, aflatoxins cause growth reduction due to protein synthesis interference (Backer *et al.*, 2005). Again these toxins have been related to several nutritional-related illnesses in humans (Backer *et al.*, 2005). Therefore, contamination of food products by aflatoxins puts consumers at high-risk health wise (Angelo *et al.*, 2009). On the contrary, Ehrilch *et al.* (2005) reported that after entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated into a less harmful aflatoxin M1 (Ehrilch *et al*., 2005). Thus, this paper reports the determination of total aflatoxins, moisture level and mould counts/isolation in groundnut (*Arachis hypogaea* L.) products in some areas of Benue State, Nigeria.

Materials and Method

Study area

Data collection was made in Makurdi and Otukpo in Benue State-Nigeria, these are municipal areas. Makurdi is incidentally the capital of Benue state. It is located between longitude 8.51ºEast and Latitude 7.74ºNorth. Makurdi has a population of 300, 377 and landmass of 8,934 square kilometers. Otukpo area has an estimated population of 160,830. And landmass of 6,891 Square kilometers (*National Population Commission of Nigeria*, 2000; Seibert, 2007). The above study areas selected lies in the tropical guinea savanna zone of the middle belt region of Nigeria. The raining season spread between April and October. The Areas has a mean annual rainfall of 1000 mm and temperature fluctuates between the minimum of 27 to 28ºC and maximum of 30 to 34ºC (*National Population Commission of Nigeria*, 2000; Eriksson and Guedes, 2006).

Sample collection

Samples of raw groundnut and processed groundnut products were collected from the two selected Area (Makurdi and Otukpo). For each of the purchased groundnut product, 5 sampling locations were earmarked in each of the areas and in turn 3 samples (at different random points) collected from each of the locations. The 3 samples were composited amounting to 1 sample per location per area for each product. To get the processed products, sample of raw groundnuts were collected from each of the locations and combined to form 1 composite per area; and the processed into paste, oil and cake. With this, a total of 54 samples were obtained. These groundnuts oils collected were transported in sterile bijou bottles while the raw groundnut, groundnut cake and groundnut paste were collected in plastic containers and transported to Benue State University Chemistry Research Laboratory for processing and analysis.

Method of processing groundnut into products

The raw groundnuts collected were dried at room temperature and then roasted. The roasted groundnuts were ground into groundnut paste using grinding machine. The paste was mixed thoroughly with warm water for about 1 h 45 min in order to extract oil from the mixture. The oil was decanted and the solid part was fried to obtain the groundnut cake.

Sample preparation

The groundnut cake and paste were grounded. The groundnut oil was extracted directly. The samples were extracted according to the general scheme of the ELISA test kit. A mass or volume of the sample were transferred to a vessel and combined with the extraction solvent. For the groundnut cake and paste, 2 – 20 g of the sample was used for analysis. Also 10-20 mL of groundnut oil was used for assay. After mixing with 100-200 mL of 80% acetonitrile for 1 min, the samples were centrifuged and the supernatants were collected for analysis (Hocking and Pitt, 1993).

Determination of moisture content

The moisture content (MC) was determined by oven drying in accordance with the AOCS method using Sartoruis MA-100 moisture analyzer (IARC, 1987).

Determination of aflatoxin in the groundnut products

The ELISA was performed according to the manufacturer's instruction (Helica Biosystems Inc, Santa Anna, CA). All reagents for ELISA were equilibrated to room temperature before use. About 200 µL of the sample was pipetted into the appropriate wells and mixed. Thereafter, 100 µL of the mixture was transferred to the appropriate antibody-coated wells in triplicate and incubated at ambient temperature for 30 min. The wells were washed with PBS-T and tapped dry. About 100 µL of aflatoxin HRP- conjugate was added to each antibody coated well and incubated at room temperature for 30 min afterward washed three times with PBS-T and tapped dry. In addition, 100 µL of the TMB substrate was added to each microwell and the plate were incubated at ambient temperature for 10 min. Finally, about 100 µL of stopped solution was added to each well. The optical density (OD) of each of the microwell was read at 450 nm on a StatFax 2100 using a differential filter of 630 nm (Hurburg, 2005). Pearson Correlation Coefficient analysis between the moisture levels and the concentrations of the aflatoxin was carried out. The results were also analyzed for statistical significance ($p<0.05$) using ONE WAY ANOVA F-ratio.

Interpretation of results on aflatoxin using Romer agra-quadrant assay method

Using Optical Density (OD) value expressed as percentage of the OD of zero (0) standard, a dose-response curve was constructed using the five standards. The amount of aflatoxin in each standard was taken as the unknown measured by interpolating from the standard curve. Results were calculated using the Romer Log/Logit spread sheer provided. The log/Logit regression model was used for results interpretation. The linearity coefficient (V2) of the calibration curve was not less than 0.985. An OD value of less than 0.5 absorbance units for 0 ppb standard may indicate deterioration of reagent (Romer, 2013).

Mould counts

5.0 g of sample was mashed and transferred in an appropriately labeled Bottle (A) containing 45 mL of peptone water and mixed thoroughly by shaking. The resultant solution was then transferred into another bottle labeled (B) containing 18.0 mL peptone water and shaken to mix. Then about 2.0 mL of the (B) solution was transferred into another Bottle (C) containing 18.0 mL of peptone water. Solution A duplicated solution B and C and they were transferred simultaneously into the marked Petri-dishes i.e. solution A into dish A and A'; solution B into dish B and B' and solution C into dish C and C'. Prepared molten Potatoes Dextrose Agar (PDA) was allowed to cool at temperature of about 48-54℃. About 10.0 cm^3 (from 50 mL) of the molten agar was smeared on the petri-dishes marked (A and A') and (B and B') gently swirled properly to mix. The remaining 10.0 mL was then emptied on the petri-dish mark (C and C') to serve as a control. The Agar was allowed to set/gel. The Petri-dishes were then incubated using ThermoFisher Scientific

Incubatorat a temperature of 37℃ for 48 h. The result was taken as colony-forming unit per milliliter.

Mould isolation and identification

In isolation of moulds associated with each the studied products (cake, paste and oil), one gram (1 g) of each sample (cake and paste) was homogenized in 9 mL of sterile distilled water. This produces a homogenate used as a stock solution of each sample. Further serial dilutions of the resultant homogenates were made to the fourth diluents. Then, 1 ml of each dilution was inoculated on sterile potato dextrose agar (PDA) in a Petri dish using the pour plate method. The oil was used directly. Inoculated plates in triplicate were sealed with paraffin wax and incubated at room temperature (25 ±2ºC) for 2-5 days with daily observation. Chloramphenicol (30 mg/mL) was added to the medium to discourage bacterial contamination. The total number of mould colonies per plate was counted using a colony counter. The number of individual mould colonies per plate was also recorded as well as the percentage frequency of each isolate (Amadi *et al.*, 2014; Stella *et al.*, 2016).

Results and Discussion

Moisture content

This particular analysis is vital because high moisture content influences growth of fungi. The results of the moisture content in the groundnut products in the various locations are presented in Tables 1 and 2. The results of moisture contents in Makurdi area is shown in Table 1. The results revealed that, the moisture levels varied from 5.45 ±0.22 to 7.65 ±0.72% for cake product, 0.71 ±0.08 to 1.35 ±0.04% for oil and 19.55 ±0.68 to 26.96 ±0.10% for paste. All the results were in the tolerable limits of 15% for cake, 2% for oil and 30% for paste, respectively (Kaaya *et al.*, 2005). The result of moisture content in Otukpo area as shown in Table 2 also revealed that the values varied from 3.90±0.37 to 7.70±0.08%, 0.72±0.03 to 0.98±0.16% and 18.86±0.12 to 26.15±0.97% for groundnut cake, oil and paste respectively. Though the result of moisture content for the pastes are higher, however all the collected samples fall within the tolerable limit of 15% for cake, 2% for oil and 30% for paste respectively as previously opined by (Kaaya *et al.*, 2005). The moisture values of the processed products were relatively lower than the purchased samples in most cases.

Table 1: Moisture content of the processed and the purchased groundnut products in Makurdi area

S/N	Sampling locations	Moisture content (wt. %)		
		Cake	Oil	Paste
1.	Processed	5.48±0.27	0.71±0.08	19.55±0.68
2.	Wadata Market	7.65±0.72	1.35±0.04	26.96±0.10
3.	Kanshio Market	7.65±0.72	0.85±0.04	26.64±0.14
4.	Rail way Market	5.90±0.51	0.93±0.07	22.57±0.41
5.	North Bank Market	7.38±0.92	1.12±0.11	24.95±0.41
6.	Wurukum Market	5.45±0.22	0.95±0.04	20.92±0.13

Table 2: Moisture content of the processed and the purchased groundnut products in Otukpo area

S/N	Sampling locations	Moisture content (wt. %)		
		Cake	Oil	Paste
1.	Processed	3.90±0.37	0.72±0.03	18.86±0.12
2.	Otukpo main market	7.70±0.08	0.84±0.80	26.13±0.86
3.	Sabon geri market	5.40±0.50	0.89±0.04	21.86±0.87
4.	OBC Junction	7.03±0.24	0.98±0.16	21.46±0.50
5.	Otukpo Hausa Qtrs	4.43±0.69	0.84±0.08	21.13±0.32
6.	Ela Market	7.13±0.60	0.89±0.11	26.15±0.97

Table 3: Total aflatoxin levelof the processed and the purchased groundnut products in Makurdi area

S/N	Sampling locations	Aflatoxin concentration (µg/kg)		
		Cake	Oil	Paste
1.	processed	0.70±0.08[a]	7.53±0.35[b]	5.50±0.21[c]
2.	Wadata Market	0.86±0.04[a]	10.06±0.12[b]	10.9±0.16[c]
3.	Kanshio Market	0.86±0.04[a]	9.75±0.33[b]	10.80±0.21[c]
4.	Rail way Market	0.73±0.04[a]	9.69±0.15[b]	8.93±0.32[c]
5.	North Bank Market	0.80±0.16[a]	10.03±0.04[b]	9.63±0.24[c]
6.	Wurukum Market	0.70±0.08[a]	9.96±0.05[b]	7.18±0.20[c]

Table 4: Total aflatoxin Level of the processed and the purchased groundnut products in Otukpo area

S/N	Sampling locations	Aflatoxin concentration (µg/kg)		
		Cake	oil	paste
1.	Processed	0.36±0.04[a]	6.86±1.16[b]	8.38±0.18[c]
2.	Otukpo main market	1.46±0.12[a]	7.80±0.38[b]	10.21±0.12[c]
3.	Sabon Geri market	0.93±0.04[a]	7.90±0.84[b]	9.63±0.78[c]
4.	OBC Junction	1.60±0.21[a]	9.10±0.74[b]	9.32±0.57[c]
5.	Otukpo Hausa Quarters	0.89±0.04[a]	7.86±0.85[b]	9.02±0.26[c]
6.	Ela Market	1.73±0.12[a]	8.53±0.95[b]	10.43±0.56[c]

Total aflatoxins level in the groundnut products

The results of the total aflatoxin levels (µg/kg) in the processed and purchased groundnut products from the various sampling locations are shown in the Tables 3 and 4. The results of TAF levels in all the studied samples were obtained and compared with the MPL set by NAFDAC (Ezekiel *et al.*, 2013; Salau *et al.*, 2017); Codex Alimentarius commission (CAC) (Ubwa *et al.*, 2014). The results for the level of total aflatoxin in Makurdi area as shown in Table 3 revealed that both the processed and the purchased groundnut products varied from 0.70±0.08 to 0.86±0.04 µg/kg for the cake samples, 7.53±0.21 to 10.06±0.12 µg/kg for oil and 5.50±0.21 to 10.90±0.16 µg/kg for the paste. Similarly, the results for aflatoxin levels in Otukpo area as shown in Table 4 revealed that the values for cake varied from 0.36±0.04 to 1.73±0.12 µg/kg, for oil 6.86±1.16 to 9.10±0.74 µg/kg and 8.38±0.18 to 10.43±0.56 µg/kg for groundnut paste. The values of the TAF in all these samples fell below the MPL of 20 µg/kg for NAFDAC (Ezekiel *et al*., 2013; Salau *et al*., 2017) and 10 µg/kg for the CAC (Ubwa *et al*., 2014) except Wadata market (oil and paste), Kanshio market paste and North bank market oil in Makurdi area; and Otukpo main market and Ela market pastes in Otukpo area that have TAF marginally above the CAC permissible limit. The variation was attributed to the handling footprint of these samples from the location. The presence of total aflatoxin could be due to the influence of mechanical damage, insect and bird damage, climatic conditions (drought, stress or excessive rainfall), soil factors on the nuts used for the processing of these products (Kaaya *et al.*, 2005; Christie *et al.*, 2010). Importantly, almost in all cases the processed samples gave lower amount of aflatoxin than the purchased groundnut products. In which case, this signifies pre-contamination of the aflatoxin in these products.

In a nutshell, the Pearson correlation coefficient (i.e. with r = 0.3439) showed there was moderate positive correlation between the moisture levels determined to the concentration of total aflatoxin in these samples. Thus, there is tendency that high moisture levels resulted into high levels of aflatoxin and vice versa for all the areas studied as it has been previously claimed (Christie *et al.*, 2010). In addition, the results of one-way ANOVA F-ratio analysis of the TAF for the cake, oil or paste confirmed there was no significant difference at $p < 0.05$ in either of the products for the respective areas. In addition, each product per study area has TAF different from the other products. The difference in the TAF is majorly the product type effect. The different products possess varying moisture levels. In addition, there was no significant difference between the processed and purchased products. Hence the

ground nut samples were infected by the aflatoxin on the farm, during storage (raw storage and final product storage) as it has been similarly reported by (Santini and Ritieni, 2013). The results on the studies of total aflatoxin levels in Groundnut (Arachis hypogaea Leguminosae) products in Makurdi and Otukpo of Benue State - Nigeria revealed that the products are unwholesome with respect to aflatoxin. Unfortunately, the consumption of food-laden with aflatoxin for long time can lead to aflatoxin poisoning (Christie, Kaaya and Kimani, 2010).The poisoning can have negative effect on the liver and other organs of the body system. It may also lead to hepatitis, liver damage among other health consequences (Christie *et al.*, 2010). It could even result into dead. In fact, aflatoxin has adverse effects on children. It could be passed from mother to the infant as well through breastfeeding (Williams, 2004). Furthermore Leszczynska *et al*. (2001) also successfully reported the use of ELISA method (which was also used in this research) for the determination of aflatoxin in food. They also confirmed that the method makes it possible to determine aflatoxin M_1 at a level of 2.5 ng/kg, aflatoxin B_1 at 12.5 ng/kg and total aflatoxin at 25 ng/kg. Hence, ELISA method is reliable, very fast and sensitive among many available methods (Leszczynska *et al.*, 2001).

Mold/fungi count

In addition, the Mold/Fungi count was determined for all the samples and the results are shown below in Tables 5 and 6. This test added credence to the results of the total aflatoxin reported above.

The results of mold/fungi count in Makurdi area (Table 5) revealed that the values for cake varied from $<1.0x10^5$ CFU/mL to $>1.0x10^5$ CFU/mL, $<1.0x10^3$ CFU/mL to $>1.0x10^3$ CFU/mL for oil and $<1.0x10^5$ CFU/mL to $>1.0x10^5$ CFU/mL for the paste. The North Bank purchased cake samples had more growth than the rest of the samples including the processed product. The growth in Wurukum purchased oil also had more growth than the rest of the collected oil sample in other of the locations. Also, the mold/fungi counts in North Bank, Wadata and Kanshio purchased paste have more growth than the others within Makurdi area. Incidentally, these locations as well had substantial levels of total aflatoxin as earlier seen. This is due to the fact that there was high moisture level in those samples (Gilbert and Anklam, 2002). In addition, these exceptionally cases in Makurdi study area may also be due to the influence of immediate environ and handling conditions as these locations are densely populated than most other locations of the study. The mold/fungi count results in Otukpo is as presented in Table 6. The results implied that, the cake result values varied from$<1.0x10^5$ CFU/mL to $>1.0x10^5$ CFU/mL,$<1.0x10^3$ CFU/mL to $>1.0x10^3$ CFU/mL for oil. The results for groundnut paste varied from $<1.0x10^5$ CFU/mL to $>1.0x10^5$ CFU/mL. The results across the locations revealed that, the mold/fungi growth in Hausa Quarters for cake samples purchased was higher than the other locations. Oil values showed that, the Ela market has more growth. Otukpo main market, Sabon geri market and Otukpo Hausa Quarters gave higher values for growth in the paste samples than the others due to high moisture level. The variation of the fungi counts of the processed product from those purchased could be attributed to the storage and processing conditions. The mold/fungi counts in general were relatively high in the groundnut paste as compared to the cake and oil samples due to the higher moisture levels in the paste samples. The fungi counts were relatively high because the sampling was carried out during the commencement of rainy season. Lack of favourable fungi growth conditions is likely the cause of low fungi count in some of the samples (IARC, 1987).

Table 5: Mold/fungi counts of the processed and the purchased groundnut products in Makurdi area

S/N	Sampling locations	Mold/Fungi counts CFU/mL		
		Cake	Oil	Paste
1.	Processed	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$<1.0 \times 10^5$
2.	Wadata Market	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
3.	Kanshio Market	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
4.	Rail way Market	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$<1.0 \times 10^5$
5.	North Bank Market	$> 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
6.	Wurukum Market	$< 1.0 \times 10^5$	$>1.0 \times 10^3$	$<1.0 \times 10^5$

Table 6: Mold/fungi counts of the processed and the purchased groundnut products in Otukpo area

S/N	Sampling locations	Mold/Fungi counts CFU/mL		
		Cake	Oil	Paste
1.	Processed	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$<1.0 \times 10^5$
2.	Otukpo main market	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
3.	Sabon geri market	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
4.	OBC Junction	$< 1.0 \times 10^5$	$<1.0 \times 10^3$	$<1.0 \times 10^5$
5.	Otukpo Hausa Qtrs	$> 1.0 \times 10^5$	$<1.0 \times 10^3$	$>1.0 \times 10^5$
6.	Ela Market	$< 1.0 \times 10^5$	$>1.0 \times 10^3$	$<1.0 \times 10^5$

Table 7: Mold/fungi isolation and identification

Genus	Species	Groundnut products			Isolate	%
		Cake	Oil	Paste		
Penicillium	*P.commune*	+	–	+	18	8.87
	A.niger	–	–	+	25	12.32
	A.flavus	+	+	+	91	44.83
Aspergillus	*A.lentulus*	–	–	+	23	11.33
	A.terreus	+	+	+	16	7.88
Rhizopus	*R.stolonifer*	+	+	+	8	3.94
Lasiodiplodia	*L.theobromae*	–	–	+	3	1.48
Fusarium	*F.moniliforme*	+	–	+	5	2.46
Mucor	*M.nidicola*	+	+	+	9	4.43
Curvularia	*C.species*	–	+	+	5	2.46

(+)Presence of growth, (-) absence of growth

Fungi isolation and identification

The results of fungi count on the cultured samples collected from the studied areas are shown in Table 7. Total of 203 isolates were recorded consisting of: *Aspergellus niger*, 25(12.32%); *Aspergellus flavus*, 91(44.83%); *Penicillium commune*, 18(8.87%); *Curvularia spp*, 5(2.46%); *Rhizopus stolonifer*, 19(3.48%); *Lasiodiplodia theobromae*, 8(1.47%); *Fusarium moniliform* 13(2.39%); *Mucor nidicola*, 9(4.43%); *Aspergellus lentulus*, 23(11.33%) and *Aspergellus terreus*, 16(7.88%). All the isolates identified have occurrencesacross the entire areas studied. Among the identified isolates, *Aspergellus flavus* growth predominated, especially in the cake and paste. Studies have shown that *Aspergellus flavus* is the main producer of aflatoxin. Aflatoxin are associated with some disease in livestock and human throughout the world (Williams, 2004). It causes cancer; hence its presences in food are of huge concern. The mycotoxins are of greatest agro-economic importance, therefore there should be concerted effort in monitoring their presence for the sake of our food safety (Hocking and Pit, 1997).

Conclusion and future work

Analysis of total aflatoxin in processed and purchased ground nut products (cake, oil and paste) in Makurdi and Otukpo areas of Benue State-Nigeria was successfully carried out. The levels of total aflatoxin in the processed and the purchased samples were within the permissible limit set NAFDAC (20 µg/kg) and CAC (10 µg/kg) except Wadata market (oil and paste), Kanshio market paste and North bank market oil in Makurdi area; and Otukpo main market and Ela market pastes in Otukpo area that have TAF marginally above the CAC permissible limit. The variation was attributed to the handling footprint of these samples from the location. The Pearson correlation coefficient (i.e. with r = 0.3439) showed there was

moderate positive correlation between the moisture levels determined to the concentration of total aflatoxin in these samples. Thus, there is tendency that high moisture levels resulted into high levels of aflatoxin and vice versa for all the areas studied as it has been previously claimed. In addition, the results of one-way ANOVA F-ratio analysis of the TAF for the cake, oil or paste confirmed there was no significant difference at $p < 0.05$ in either of the products for the respective areas. In addition, each product per study area has TAF different from each other product. The difference in the TAF is majorly the product type effect. The different products possess varying moisture levels. This is a major condition forthe growth of fungi. Mold/fungi count results showed that aflatoxin causative organism *Aspergellus flavus* was prevalent (i.e. with 44.83% level of occurrence) for all the areas. Therefore, these products both processed and purchased were contaminated with aflatoxin due to especially mechanical damage, insect and bird damage, climatic conditions (drought, stress or excessive rainfall), soil factors of the nuts used for processing these products. Hence the ground nut samples were possibly infected by the aflatoxin on the farm and as well as during storage (raw storage and final product storage). Therefore, it's appropriate to keep monitoring the presence of aflatoxin in groundnut products in order to avoid its poisoning.

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Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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