Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Quantitative Appraisal of Total Aflatoxin in Ready-to-eat Groundnut in North-central Nigeria

Funmilola Sherifat Adefolalu[1], Daniel Ojochenemi Apeh*[2], Susan B. Salubuyi[1], Mary Galadima[1], Augustine Agbo[1], Michaella Onyinye Anthony[1], Hussaini Anthony Makun[1]

[1] *Department of Biochemistry, School of Life Sciences, Federal University of Technology Minna, Nigeria*

[2] *Department of Biochemistry, Faculty of Natural Sciences, Kogi State University Anyigba, Nigeria*

(Received: 7 October 2020 Accepted: 24 May 2021)

KEYWORDS

Total aflatoxin;
Processed groundnut;
North-central Nigeria

ABSTRACT: Following the CODEX Alimentarius Commission's request for data to aid decision-making in the review of regulated limits of mycotoxin in groundnut, this study determined the incidence of total aflatoxin (AFT) in processed groundnut from Niger state, which is located in Nigeria's north-central region. A total of 180 ready-to-eat groundnut samples were collected across four microclimatic zones in Niger state, with 60 samples each of boiled groundnut, roasted groundnut, and groundnut cakes. The ELISA technique was used to test the samples. For groundnut cakes, roasted groundnut, and boiled groundnut, the incidence and mean concentrations of AFT were 100% (11.15±3.31 μg kg^{-1}), 83.3% (4.50±2.47 μg kg^{-1}) and 38.3% (1.51±2.13μg kg^{-1}) respectively, across all areas, suggesting that groundnut cake had the highest incidence and concentrations of AFT. While, 95% of groundnut cake, 53.3% of roasted groundnut, and 18.3% of boiled groundnut samples had AFT levels above 4μg kg^{-1}. The result of this research suggests that storage time had a negative effect on the safety of groundnut.

INTRODUCTION

Groundnut (*Arachis hypogaea*) commonly called peanut, goober, pindar or monkey nut, is a legume crop cultivated mainly for its edible seeds in some regions of the world. After China, India and the United States, Nigeria is the 4th largest groundnut producer worldwide and leading producer in Africa [1]. Groundnut is consumed in various forms; boiled, roasted with or without shell, as cake/flakes (kulikuli), as oil, and even soup among other uses. The economic value of groundnut as a commodity for inter-continental trade has prompted discussions, which aim at reviewing the current maximum residue level (MRL) of aflatoxins, previously set by the WHO/FAO Joint Expert Committee on Food Additive and Contaminant (JECFA) [2]. The 41st session of the CODEX meetings chaired by India Electronic Working Group has attempted to review this MRL, hence proposing 10 μg kg^{-1} [2]. It is therefore justifiable that countries that have a high production and export potential such as Nigeria should not be left out in making data available for such a critical policy decision.

Aflatoxins are secondary metabolites of mainly *Aspergillus flavus* and *Aspergillus parasiticus* fungi, which have been associated with child stunting, synergistic harmful effect with hepatitis B virus infection and acquired immune deficiency syndrome [3]. They also exhibit acute toxicity manifestations such as nausea, diarrhoea, and hepatotoxicity, and have been implicated in hepatocarcinogenesis, as such aflatoxins have been classified by IARC as Group 1 carcinogens [4]. Groundnut

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*Corresponding author: danapeh@gmail.com (D. Ojochenemi Apeh)
DOI: 10.22034/jchr.2021.1911495.1196

quality is majorly affected by its high susceptibility to fungal attack especially by the aflatoxin-producing molds; *A. flavus* and *A. parasiticus* which produce aflatoxins in the kernels of groundnuts especially during storage, and sometimes on field [5].

Groundnut as a major export product from Nigeria suffers border rejection from EU countries due to the presence of mycotoxins such as aflatoxins, hence leading to negative economic impact. In light of the prioritized positioning of Nigeria in the global groundnut production map, as well as its high local consumption, and export value, this research is aimed at providing AFT incidence and concentration data of ready-to-eat (RTE) groundnut within North-central Nigeria.

MATERIALS AND METHODS

Sample Collection

A total of 180 groundnut samples were collected across Niger state in North-central Nigeria, with 60 each of boiled groundnut, roasted groundnut, and groundnut cake. The samples represent 15 Local Government Areas (LGAs) and can be further grouped according to the annual rainfall (mm) into four microclimatic zones; Zone 1 (Suleja and Tafa) have annual rainfall of 1400 mm, zone 2 (Borgu and Magama) have annual rainfall between 1200-1400 mm, zone 3 (Agaie, Bosso, Gurara, Katcha, Lapai, Lapai, Minna, Munya, Shiroro, Paiko) have annual rainfall between 1000-1200 mm, and zone 4 (Rafi and Wushishi) have annual rainfall below 1000mm.

Sample preparation and Aflatoxin extraction

Using a Romer series II Mill, the samples were pulverized and thoroughly mixed. Each powdered sample was weighed into a clean container, which was then filled with 25 ml of 70:30 (v/v) methanol-water extraction solution and sealed. The sample was vigorously shaken for 3 minutes using a shaker at 250 rpm, allowed to settle, and then filtered using Whatman No. 2 filter paper to collect the filtrate.

Aflatoxin Analysis Using Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay is based on the antigen-antibody reaction. The extracted sample and enzyme-conjugated aflatoxin were mixed and added to the antibody coated micro wells. AFT in the sample and control standard were then allowed to compete with enzyme conjugated aflatoxin for the limited antibody binding sites. After washing, an enzyme substrate was added until a blue colour was observed. Blue/green-bordered dilution strips were placed into a micro-well strip holder. One dilution well was required for each standard, (0, 5 10, 20, 40 µg kg^{-1}) and sample. An equal number of antibody coated micro-well strips was placed in a micro-well strip holder. Conjugate (200 ul) was pipetted from the green-capped bottle and placed in a separate test tube. Using an 8-channel pipette, 200 ul of the conjugate was dispensed into each blue/green-bordered dilution well. One hundred microliter (100 ul) of each standard or sample was pipetted and added into the appropriate dilution well containing 200 ul of conjugate. Each well was mixed by carefully pipetting it up and down 3 times and immediately 100 ml of the contents from each dilution well was transferred into a corresponding antibody coated micro-well. The solution was incubated at room temperature for 15 minutes**.** Each of the contents of the micro-well strips was emptied into a waste container/ sink, washed by filling each micro-well with distilled or de-ionized water to remove those that did not bind to the antibody coated wells. This was repeated 4 times for a total of 5 washes. The required amount of substrate from the blue-capped bottle was measured (~120 ul/well or 1ml/strip) and dispensed into a separate container (reagent boat for an 8-channel pipette). The substrate (100 ul) was pipetted into each micro-well strip using an 8-channel pipette, and incubated at room temperature for 5 minutes after which a blue color developed. The required amount of stop solution from the red-capped bottle was measured (~120ml/well or 1ml/strip) and dispensed into a separate container (e.g. reagent boat for an 8-channel pipette). The intensity of the color is inversely proportional to the concentration of aflatoxin in the sample. A stop solution was then added which changed the color to yellow.

The micro well strips are measured optically using a STAT FAX Elisa Reader MODEL: 303 PLUS with absorbance filter of 450 nm. The limit of detection determined by the average values of 10 aflatoxin-free samples plus 2 standard deviation was 3 ppb. The limit of quantification described as the lowest concentration point on the calibration curve that this test can reliably detect aflatoxin was 4ppb. The percentage recovery for spiked groundnut sample was 92±2%. These was achieved based on methodology adopted from Apeh et al. [6] and Onyedum et al. [7]

Statistical analysis

IBM SPSS 22.0 software was employed to calculate the mean and standard deviation, while MsExcel was used to plot figures.

RESULTS AND DISCUSSION

The results of the analyses of AFT are shown in Table 1, and Figures 1 and 2. Table 1 shows the mean ± standard deviation, concentration range, number of positive samples above EU ML of 4 µg kg^{-1} taking into consideration the various forms of processed groundnut. The result indicates that all the processed groundnut types but not all samples (caked, roasted and boiled) across the zones analyzed, were contaminated with AFT at detectable levels. While Figure 1 clearly shows that across the four zones, the concentrations of AFT in groundnut cake was consistently highest than other processed form, Figure 2 indicates that groundnut cake presented the highest incidence of AFT. The concentrations of AFT in roasted groundnut was second to groundnut cake when both mean concentration level and incidence were considered, as such it was higher than the AFT level detected in boiled groundnut, and its incidence.

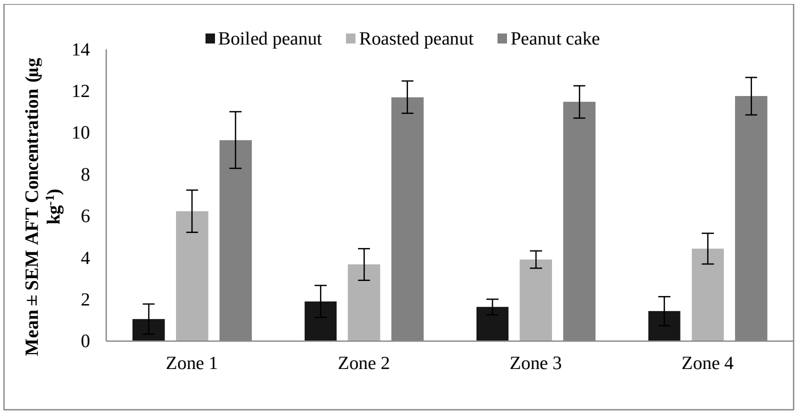

Figure 1. Mean concentration (µg kg^{-1}) of AFT in processed peanut across the microclimatic zones of Niger State, North-central Nigeria

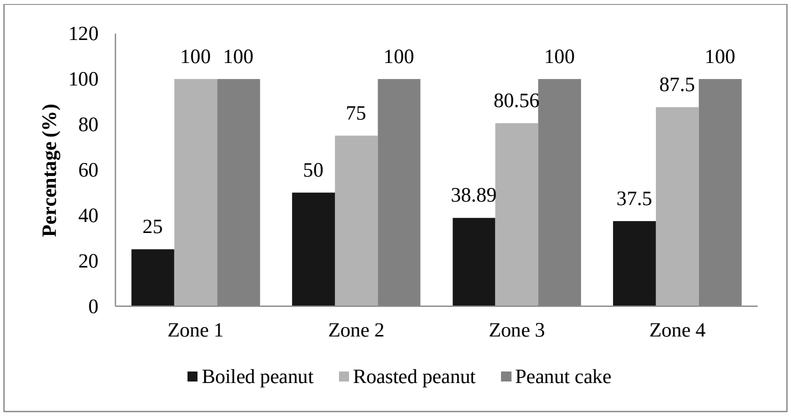

Figure 2. Incidence (%) of AFT in processed peanut across the microclimatic zones of Niger State, North-central Nigeria

From Table 1, it was observed that boiled groundnuts had the least contamination levels with values ranging between 2.60 to 8.90 µg kg^{-1}. Only 18.3% of the samples across all microclimatic zones were found to contain aflatoxins above

EU's ML (4 μg kg^{-1}). It is worthy of note that the groundnuts that are boiled for consumption are usually fresh groundnuts that has spent zero to a few weeks in storage. An analysis carried out by Obi *et al.* [8] on aflatoxin in boiled groundnut in Nnewi, Nigeria showed 42% contamination of boiled groundnut during the wet season. This is possible because lack of sufficient sunlight for drying of groundnut pods after harvest exposes it to suitable water activity for fungi proliferation during wet season. The common practice is for teenagers and adults to hawk boiled groundnut for sale during the short period in which groundnut is harvested, after which the bulk of harvest are air dried. In cases where groundnuts are not contaminated on field, they are expected to show less contamination when boiled immediately after harvest. Hence, storage time and method play a crucial role in the contamination of groundnut. This also is shown in the findings of Baributsa and colleagues [9] in a research in which they discovered that storing groundnut in Purdue Improved Crop Storage (PICS); a hermetic triple layer bag completely prevented the groundnuts from infestation and production of mycotoxins as opposed to their counterparts stored in woven bags.

Table 1. Incidence and concentrations of total aflatoxin in ready-to-eat groundnut

Location	**Boiled groundnut**				**Roasted groundnut**				**Groundnut cake**			
	n/N	Mean±SD (μg kg^{-1})	Range (μg kg^{-1})	n above 4μg kg^{-1} (%)	n/N	Mean±SD (μg kg^{-1})	Range (μg kg^{-1})	n above 4μg kg^{-1}(%)	n/N	Mean±SD (μg kg^{-1})	Range (μg kg^{-1})	n above 4μg.kg^{-1} (%)
Zone 1	2/8	1.06±2.05	3.20-5.30	1 (12.5)	8/8	6.24±2.86	3.10-10.60	5(62.5)	8/8	9.65±3.84	4.30-14.70	8(100)
Zone 2	4/8	1.91±2.18	2.60-5.10	2(25)	6/8	3.39±2.49	2.90-6.80	3(37.5)	8/8	11.71±2.22	7.90-13.70	8(100)
Zone 3	14/36	1.64±2.28	2.70-8.90	7(19.4)	29/36	3.92±2.45	2.70-9.20	18(50)	36/36	11.48±4.61	2.90-19.80	33(91.7)
Zone 4	3/8	1.44±1.99	3.50-4.20	1(12.5)	7/8	4.44±2.06	3.80-7.30	6(75)	8/8	11.76±2.56	7.70-15.40	8(100)
Total	23/60 (38.3%)	1.51±2.13	2.60-8.90	11 (18.3)	50/60 (83.3%)	.4.50±2.47	2.70-10.60	32 (53.3)	60/60 (100%)	11.15±3.31	2.90-19.80	57 (95)

Key: n= number contaminated with AFT, N= number analysed. 0/60 boiled groundnut sample was above CODEX proposed ML of 10 μg kg^{-1}, 1/60 roasted groundnut sample was above CODEX proposed ML, 38/60 groundnut cake samples were above CODEX proposed ML of 10 μg.kg^{-1}On the other hand, roasted groundnut is processed from dried, or stored groundnut, the duration of storage varies and hence can influence the levels of fungi contaminant in the nuts. As a result, the roasted groundnuts presented higher levels of aflatoxin contamination than boiled groundnut. The result showed that roasted groundnut samples across all sampling zones had aflatoxin presence in concentrations ranging from 2.70 μg kg^{-1} to 10.60 μg kg^{-1} with about 53.3% of all samples containing aflatoxins above EU's maximum permissible limit (MPL). This result compliments the findings of Bankole and his colleagues who detected aflatoxin B_1 (AFB_1) in 64% of dried roasted groundnut out of which 32% were contaminated above MPL [10].

In a research to determine the effect of processing on the fungal counts and aflatoxin presence in roasted bambara groundnut in storage, it was reported that roasting bambara nut seeds at temperatures up to 140°C for at least 20 minutes degraded aflatoxins [11]. However, the elimination of fungal contaminants in the seeds was not complete and the "leftovers" went on to produce aflatoxins in storage. This also applies to roasted groundnuts, since even though they are roasted at temperatures high enough to induce aflatoxin degradation, if aflatoxin-producing fungi are not completely eliminated, storing the roasted groundnut for an extended period of time in conditions that favor fungal activity can result in a substantial increase in aflatoxin concentration over time. Groundnut cake had the highest contamination rate having values ranging between 2.90 μg kg^{-1}and 19.80 μg kg^{-1} with 95% of all samples containing aflatoxins above EU's MPL. Groundnut cake also called ‘kulikuli’ in Nigeria is a product of further processing of roasted dry groundnut and as such, the raw material (dry groundnut) may have been stored for sufficient time for mold development. Hence, groundnut cakes face a higher risk of contamination than the other forms in which groundnut is consumed. This result supports the findings of Hao and Ann [12] who isolated the toxigenic mold, *A. Flavus* from 100% of their samples. The result of this research is consistent with the findings of related researches in other parts of the world. In Ethopia, 77.5% of groundnut samples was reported to be contaminated with aflatoxins within the range of 15 – 11900 $\mu g.kg^{-1}$ [13]. Lower incidence was reported in Turkey where 19.2% of groundnut were contaminates with aflatoxins and ranged from 0.16 to 60.9 $\mu g\ kg^{-1}$ [14], and also in Sudan where AFB_1 in groundnut oil occurred in only 3.57% samples and the mean value was 0.6 $\mu g\ kg^{-1}$ [15].

Water activity refers to the amount of water available for biochemical activities and growth of fungal and bacterial species in food [16]. It is measured within the range of 0-1. Food products with high water activity (above 0.65) are at high risk of fungal activities resulting in mycotoxin production [17]. In previous research [11], it was discovered that (*Vigna subterranea (L.)Verdc*) at 140^0C for 20 minutes had water activity less than the original water activity of their non-roasted counterpart and also had a low microbial load; hence minimal aflatoxin contamination was found present. However, when the roasted nuts were kept in storage, there was a progressive and significant increase in the water activity of the nuts with values up to 0.95 at day 10 and values even greater than the absolute value of 1.0 at days 15 and 20. At this level, *A. flavus* which grows at a_w=0.87- 0.99 [18] finds a favorable condition for metabolic processes resulting in the production of aflatoxins. This implies that despite the heat treatment by roasting the bambara nut seeds, increased storage time significantly increases the risk of aflatoxin production. The same assertion was inferred by Waliyar et al. [19]. Apart from fungi infection, an in vitro xylem sap experiment proved that groundnut plant roots can absorb AFB_1, and transport same to aerial plant parts via the xylem. Hence, groundnut seed possess the ability to be contaminated by AFB_1 from soil uptake through xylem tissue in addition to fungi infection [20].

The processing of groundnut cake (“kulikuli”) also involves a frying step involving the use of unrefined groundnut oil which could be contaminated by aflatoxin [15]. In 2010, a research team led by Elzupir *et al.* [21] found aflatoxins in 98% of their oil samples with values ranging between 0.43 $\mu g\ kg^{-1}$ and 339.9 $\mu g\ kg^{-1}$ with an average concentration of 57.5 $\mu g\ kg^{-1}$ against EU’s acceptable limit of 20$\mu g\ g^{-1}$. In older studies [19, 22 and 23], large concentrations of aflatoxins in vegetable oils at levels above MPL were also discovered. As a result, groundnut cake poses a greater risk of aflatoxin contamination than other groundnut products.

CONCLUSIONS

Based on the findings of this research, groundnut cake had the highest incidence and mean concentration of total aflatoxin, roasted groundnut followed, and boiled groundnut had the least levels. Groundnut cake, roasted groundnut, and boiled groundnut had total aflatoxin incidences of 100%, 83.3% and 38.3% respectively, and of the contaminated samples, 95% of groundnut cake, 53.3% of roasted groundnut, and 18.3% of boiled groundnut samples had total aflatoxin concentrations above the EU/Nigerian maximum permissible limit of 4 $\mu g\ kg^{-1}$. This result then suggests that there is a need to adopt good post - harvest practices, such as proper storage methods, since the findings show that longer storage duration is a supporting factor for aflatoxins contamination of groundnut.

Conflict of interest

The authors declare no conflict of interest

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