ASSESSMENT OF AFLATOXIN AND HEAVY METAL LEVELS IN SOYBEAN AND GROUNDNUT SAMPLES OBTAINED FROM MARKETS IN MAKURDI METROPOLIS

Supported by tetfund

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Received: May 09, 2024 Accepted: June 25, 2024

Abstract: Food samples containing aflatoxins and heavy metals have been responsible for the contamination of the food chain. This research aims to determine the concentration of aflatoxins and some heavy metals (Ni, Zn, Pb, Cd) contamination in soybean and groundnut food samples (widely consumed in the study area) from four (4) markets in Makurdi metropolis to monitor food contamination. Aflatoxins and heavy metals concentrations were analysed on the aforementioned samples using the Enzyme - Linked Immunosorbent Assay (ELISA) and Atomic Absorption Spectrophotometer (AAS) techniques respectively. Total aflatoxins were not detected in soybean samples while concentrations in groundnut samples ranged between 0.35±0.07-3.30±0.14 ppb. The heavy metal concentrations in Zn, Cd, and Ni in soybean were 3.29±0.04-5.01±0.05 mg/Kg, 0.03±0.00-0.06±0.04 mg/kg and 0.04±0.00-0.28±0.00 mg/kg respectively. Those of groundnut were 2.46±0.02-2.82±0.00 mg/kg, 0.03±0.01-0.04±0.01 mg/kg and 0.02±0.01-0.13±0.01 mg/kg respectively. Pb was not detected in all the samples. Taken together, this study has thus shown that aflatoxins were within the European Union and National Agency for Food Drug Administration and Control (NAFDAC) acceptable limit and heavy metal content in all samples were within the Codex Alimentarius Commission acceptable limits. This implies that the consumption of soybean and groundnut from the markets are safe as far as these two (2) contaminants are concerned.

Keywords: heavy metals, aflatoxins, soybean, groundnut, contaminants, concentration.

Introduction

Food crops have been the primary source of calories and protein for a large portion of the global population, particularly in developing nations (Schönfeldt & Hall, 2012). The prominent staple foods include; cereals, tubers, legumes and their products (Eriksson *et al.*, 2018). Grains like rice, sorghum, millet and maize, as well as tubers like cassava, yam, and sweet potatoes, are the staple foods in Africa (Haggblade *et al.*, 2017). However, because animal protein is insufficient to feed the continent's expanding population, plant protein sources such as soybean, groundnut, and sunflower are substituted for animal protein (Parisi *et al.*, 2020).

Soybeans and groundnut's high dietary values, taste, affordability and huge availability, have made many low-income families embrace them. Therefore there is increased awareness, demand and also consumption of these products in many parts of the world (Okwori *et al.*, 2010). These oilseeds are relatively cheap sources of protein and oil for humans; they also serve as feeds for animals (Al-seeni, 2012). Being legumes, they perform a huge role as food for man and feeds for animals, especially in the third world nations, where they meet as much as two-third of human nutritional needs (Gupta *et al.*, 2018). With increased cultivation and consumption of soybean and groundnut (Raj *et al.*, 2023), it is necessary to examine the quality of these oilseeds, as quality decreases with increasing concentrations of toxic substances such as aflatoxins, heavy metals, nitrates and pesticide residues which are usually introduced by various causes and processes into these oilseeds (Osman *et al.*, 2014).

Aflatoxins and heavy metals are potential environmental contaminants with the capacity to cause serious human and health problems (Eskandar & Pakfetrat, (2014). Heavy metals are not biodegradable, have long biological half-lives and have the potential to accumulate in different body organs, leading to unwanted side effects (Sarubbo *et al.*, 2015). About 25 % of the world's crops are contaminated with mycotoxins, most of which are aflatoxins (Winter & Pereg, 2019). Detecting these hazardous substances in dietetic crops of humans and animals is of significance. Dietary consumption of contaminated oilseeds can pose a risk to humans and animals as these environmental contaminants affect the wholesomeness of the food and feed (Eskandari & Pakfekrat, 2014; Thielecke & Nugent, 2018; Guchi, 2015). In this study, we investigated heavy metals such as Nickel (Ni), Cadmium (Cd), Lead (Pb) and Zinc (Zn) and the total aflatoxins contamination levels in groundnut and soybean from four (4) different markets where they are traded in Makurdi metropolis.

Experimentals

Sample collection and preparation

Groundnut and soybean samples were collected from four (4) different market places within Makurdi: Wadata, Northbank, Wurukum, and Modern markets in sterilized polythene bags. These samples were oven dried at 105 ^{o}C for 8 h, cooled to room temperature and pulverized using porcelain mortar and pestle to powder. The pulverized samples were kept in different small labelled crucibles.

Ash content determination

The ash content was carried as described by Bayero *et al.*, (2019). An amount of the sample (2 g) was ashed in an NEY M-525 Furnace for 8 h at 600 ^{o}C. The ash content of the samples was estimated using equation (1).

$$Ash\ content = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100 \qquad (1)$$

Heavy Metals Determination

With modification, the method described by Akinyele & Shokunbi (2015) was adopted. The ash obtained was digested to a solution with 5 mL of 5 M HNO_3. The mixtures were filtered into 50 mL standard volumetric flasks using Whattman No 1 filter papers, and the filtrates were made to the mark with distilled water. Filtrates were used to determine the heavy metals concentration through A PG-990 Atomic Absorption Spectrophotometer. The calibration standard curve provided the basis to quantify heavy metal concentration.

Extraction for total aflatoxin

The method described by Ahmed *et al.,* (2019) was adopted with little modification**.** 5 g of each blended sample was weighed into reagent bottles and 25 mL of 70:30 methanol-water solutions was poured into each of the bottles to dilute the samples. The samples were then vigorously shaken using orbital shaker at 250 rpm for 10 min to extract toxins. The entire extracts were filtered using Whattman No 1 filter papers into centrifuge tubes and the filtrates were used for immunoassay.

Quantitative Analysis

The method described by Ahmed *et al*., (2019) was adopted. Antibody-coated microwells were inserted into a microwell holder and 300 µL of conjugate, followed by the addition of 100 µL of filtrate from the centrifuge tube were transferred into each of the coated microwells using a micro pipette, and were mixed by pipetting it up and down three times carefully, followed by incubation at room temperature for 15 min. The microwells' contents were washed away five times using buffer solution to remove any unbound toxin and then tap-dried using tissue paper. 100 µL of substrate was added to each well and the holder was shaken carefully to mix contents. Incubation for another 5 min at room temperature followed in order to develop a blue colour. The blue reaction was stopped by adding stop solution which changed colour to yellow. Using the ELISA reader, the absorbance was measured by loading the microwells in the reader in order to determine concentration of total aflatoxin in samples.

Statistical Analysis

Duplicate determination of aflatoxin and heavy metal concentrations in soybean and groundnut samples were carried out, mean values ± standard deviations were also calculated for the results and the means were statistically compared using One-way analysis of variance (ANOVA) by SPSS-statistics (version 22.0).

Results and Discussion

Table 1: Aflatoxin concentrations in soybean and groundnut in Makurdi metropolis

Study Location	Aflatoxin (ppb)	
	Soybean	Groundnut
Wurukum market	N.D	$3.00^{d}\pm0.00$
Wadata market	N.D	$0.35^{b}\pm 0.07$
Northbank market	N.D	$0.85^{c}\pm0.07$
Modern market	N.D	$3.30^{a}\pm0.14$

KEY: ± means SD of duplicate determinations, N.D: Not detected, P-Values <0.05, Mean with identical superscripts within a column are not significantly correlated

The results of aflatoxins concentration in soybean and groundnut samples from the four (4) markets (Wurukum, Wadata, Northbank and Modern) as presented on Table 1 shows that aflatoxins were not detected in soybean in all the four (4) markets. This may possibly be due to good pre- and post-harvest practices. This observation is similar to the study done by Dharmaputra, (2002) in Indonesian on soybean meal which revealed no contamination in soybean grain. However, Daga *et al*., (2015) in Santa Catarina State reported average value of contamination in soybean meal to be 1.3 ppb. About two decades ago in Egypt, El-kady and Youssef (1993) detected aflatoxin in 35 % of soybean samples in the range of 5-35 ppb. On the order hand, the values of aflatoxins contamination in groundnut samples were highest in Modern market samples and lowest in Wadata market samples, having concentrations of 3.30±0.14 ppb and 0.03±0.07 ppb respectively. This is within the Maximum Acceptable Limit (MAL) recommended by the European Union (4 ppb) and National Agency for Food Drug Administration and Control (NAFDAC) (20 ppb) (Salau *et al*., 2017). This concentration disagrees with a previous research by Elzupir *et al*., (2010) which reported high total aflatoxins levels of 0.43- 339.9 ppb in Sudan. The concentrations of aflatoxins in the research are lower as compared to previous study and this could be attributed to good hygiene in the production of crops, which includes harvesting, drying, and storage.

From this study, it is observed that the concentration of aflatoxin in groundnut is high compared to soybean which no aflatoxin was detected. This can be attributed to the fact that groundnut is more susceptible to physical damage during uprooting using hoes, causing damage to both shell and seeds. The observation could also be attributed to the fact that soybean seeds grow above the ground while groundnut seeds grow below the ground. During harvest, groundnut seeds are harvested with some soil particles attached to them. These soil particles may contain fungus, thus making them contaminated. These groundnut seeds are then carried with these contaminated soils particles into storage facilities.

It is observed that there exists a significant difference in the concentrations of aflatoxins in groundnut between the four (4) markets ($p< 0.05$). This could be as a result of the level of moisture content and post-harvest storages in hot humid climate, resulting in the growth of moulds. This present study agrees with that of Onyeke *et al.,* (2017) which total aflatoxins content of samples foods differed among three different markets in Nsukka, Nigeria. However, it disagrees with result obtained by Asemave *et al.*, (2019) which showed no significant difference at P<0.05 in products for the respective areas.

Table 2: Heavy metals concentrations in soybean in Makurdi metropolis

Study location	Cd (mg/L)	Ni (mg/L)	Pb (mg/L)	Zn (mg/L)	Ash content (%)
Wurukum market	$0.06^{a}\pm0.04$	$0.04^{b}\pm0.00$	N.D	$4.77^{d}\pm0.28$	4.00
Wadata market	$0.03^{a}\pm0.01$	$0.28^{e}\pm0.00$	N.D	$3.29^{c}\pm0.04$	6.50
Northbank market	$0.03^{a}\pm0.00$	N.D	N.D	$5.01^{d}\pm0.05$	3.00
Modern market	$0.03^{a}\pm0.01$	$0.06^{c}\pm0.01$	N.D	$3.37^{c}\pm0.02$	4.50

KEY: ± means SD of duplicate determinations, N.D: Not detected, P-Values <0.05, Mean with identical superscripts within a column are not significantly correlated

Table 2 shows heavy metals concentrations and ash content in soybean from four (4) different markets in Makurdi metropolis. The values obtained from the analysis show Zn in Wurukum, Wadata, Northbank and Modern markets to be 4.77±0.28 mg/kg, 3.39±0.04 mg/kg, 5.01±0.05 mg/kg and 3.37±0.02 mg/kg respectively. Cd concentrations were 0.06±0.04 mg/kg, 0.03±0.01 mg/kg, 0.03±0.00 mg/kg and 0.03±0.01 mg/kg; which is within the MAL recommended by Codex Alimentarius Commission (0.1 mg/kg). Ni concentrations were 0.04±0.00 mg/kg, 0.28±0.00 mg/kg, not detected and 0.06±0.01 mg/kg respectively. Pb was not detected in all locations. It was observed that Zn had the highest concentrations and Cd the least. This is similar to results obtained by Angelova *et al.*, (2003) which reported that the mean concentration of Zn was 58.4 mg/kg and Cd, 1.025 mg/kg. According to previous studies, uptake and translocation of Cd and Zn in plants are related to each other in an inverse relation (Sadrabad *et al*., 2018). It is also observed that there is no significant difference in the concentrations of Cd in soybean in the markets ($P > 0.05$). This could be attributed to the continuous use of phosphate fertilizer in the production of soybean. There is a significant difference between the concentration of Ni in soybean in Wurukum, Wadata and Northbank ($P < 0.05$). This could be attributed to the use of phosphate fertilizer in varying concentrations, this causing soybean to accumulate Ni in varying concentrations. It is observed that there is no significant difference in Zn concentrations in soybean from Wurukum and Northbank markets and also between Wadata and Modern markets ($P > 0.05$) but there exit a significant difference between Wurukum and Northbank markets and Wadata and Modern markets ($P < 0.05$). This is possibly due to soybeans from Wurukum and Northbank being cultivated in same location, and Wadata and Modern markets in same location. Thus causing the use of biosolids in varying concentrations during cultivation and thus crops accumulating. Zn in varying concentrations.

Ash content ranged from 3.00 - 4.48 % with Wadata market having the highest percentage and Northbank market the least. Faghohun *et al*., (2012) opined that ash content is a reflection of the amount of mineral element present in crops. Therefore, high ash content suggests high mineral content, as the nutritional quality of samples is improved. However, Ukam *et al*., (2008) noted that it may be reverse if it contained toxic metals which also contribute to the percentage ash content. This is similar to the findings of this research, as ash content was inversely related to the cumulative mean of heavy metals in each sample.

Table 3: Heavy metals concentrations in groundnut in Makurdi metropolis

Study Location	Cd (mg/L)	Ni (mg/L)	Pb (mg/L)	Zn (mg/L)	Ash content (%)
Wurukum market	$0.04^{a}\pm0.00$	$0.04^{b}\pm0.01$	N.D	$2.67^{ab}\pm0.07$	4.00
Wadata market	$0.04^{a}\pm0.01$	$0.02^{a}\pm0.01$	N.D	$2.82^{b}\pm00$	1.00
Northbank market	$0.04^{a}\pm0.01$	$0.13^{d}\pm0.01$	N.D	$2.46^{a}\pm0.02$	2.50
Modern market	$0.03^{a}\pm0.01$	$0.05^{bc}\pm0.01$	N.D	$2.67^{ab}\pm0.18$	1.50

KEY: ± means SD of duplicate determinations, N.D: Not detected, P-Values <0.05, Mean with identical superscripts within a column are not significant

Table 3 shows the heavy metal concentration and ash content in groundnut from the four (4) markets. The values obtained from the analysis shows Zn in Wurukum, Wadata, Northbank and Modern markets to be 2.67±0.07 mg/kg, 2.82±0.00 mg/kg, 2±46±0.02 mg/kg and 2.67±0.18 mg/kg respectively. Cd concentrations were 0.04±0.00 mg/kg, 0.04±0.01 mg/kg, 0.04±0.01 mg/kg and 0.03±0.01 mg/kg; which is within the MAL recommended by Codex Alimentarius Commission (0.1 mg/kg). Ni concentrations were 0.04±0.01 mg/kg, 0.02±0.01 mg/kg, 0.13±0.01 mg/g and 0.05±0.01mg/kg respectively. Pb was not detected in all locations. Zn had the highest concentration with Cd having the least. Difference in the concentrations could be as a result of their inverse relationship. It is also observed that there is no significant difference in the concentrations of Cd in groundnut in the markets ($P > 0.05$). This could be attributed to the continuous use of phosphate fertilizer in the production of soybean.

It is observed that there is a significant difference in the concentration of Ni in groundnut between Northbank and the other three markets ($P < 0.05$). This could be from the farm, as possible contamination may arise during the production. Contamination may be due to the use of phosphate fertilizer in the production of the groundnut from Northbank compared to other markets and also the use of waste water for irrigation. Another possibility could be from the run-off of waste water into farm where groundnut from Northbank was cultivated. There a significant difference in Zn concentrations in groundnut from Wadata, Northbank and the other two markets ($P < 0.05$). This could possibly be as a result of groundnut from Wurukum and Modern markets being cultivated at same location, and others at different locations. Thus, causing the use of biosolids in varying concentrations during cultivation and thus crops accumulating Zn in varying concentrations.

Ash content in groundnut ranged from 1.00 % from Wadata to 4.00 % from Wurukum. The result at Wadata market, Northbank market amd Modern market is close to a work reported by Alhassan *et al*., (2017) who reported in the range of 1.2-2.3%

Conclusion

This study has shown that groundnut seeds from the four (4) markets were contaminated by aflatoxin especially modern market whereas, no aflatoxin was detected in the soybean. Though the contamination of groundnuts were within the acceptable limit for both NAFDAC (20 ppb) and European Union (4 ppb) implying less negative effect on human beings except after a prolong consumption where there may bio-accumulate. This study has also shown that soybean and groundnut were contaminated by heavy metal in all the markets except Pb that was not detected in all the samples and Ni in soybean from Northbank. The extent of contamination was within Codex Alimentarius Commission and also low as compared to other studies. Result implies that the consumption of soybean and groundnut from the markets do not lose a health threat to the population in the study area. However, the periodic monitoring of these contaminants on these samples in these study areas is advocated to detect whenever they rise beyond acceptance level.

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