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EVALUATION OF THE AFLATOXIN CONTENT, ANUTRIENTS LEVEL AND PH OF SELECTED FOOD ITEMS CONSUMED IN THE SOUTH EASTERN NIGERIA

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Abstract:

This study evaluated the aflatoxin content and anutrient composition as well as the pHof different food materials consumed in South Eastern, Nigeria. Seven food materials: oil bean, melon, peanut butter, cassava chips, pepper, corn pap and African nutmeg were obtained from different parts of the South Eastern, Nigeria. The samples were processed by pulverizing into powdered form using sterile blender. The samples were then collected separately into sterile screw capped Duran bottles for usein the analysis of aflatoxin, anutrients and pH profile. The results showed that aflatoxin contentsranged from 11.027±0.5 µg/Kg in peanut butter to 2.566 ±0.2µg/Kg in cassava chips. Among the anutrients studied, highest flavonoids concentration was found in pepper (13.2 ±1.5%) peanut butter and the least in cassava chips (1.3 ±0.3%). On the other hand,Saponin content ranged from 17.3 ±1.4% in melon to 10.1±0.8 % in peanut butterwhile tannins was present only in melon and African nutmeg. However, pH ranged from 7.21 to 4.57 in melon and corn pap samples respectively. This study reveals aflatoxin contamination of all food samples studied and a relationship between the pH of food andextent of aflatoxin infestation.

Key words: *Aflatoxin, anutrient, oil bean, melon, peanut butter, cassava chips, pepper, corn pap and African nutmeg.*

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INTRODUCTION:

Aflatoxins are toxic metabolites produced by fungi, especially for saprophytic moulds growing as form of defense for organisms which can cause a wide range of acute and chronic effect [1]. According to [2], the word aflatoxin was coined in 1962 during the aftermath of an unusual veterinary crisis near London England, at which approximately 100,000 turkeys died. The mysterious turkey disease was linked to groundnut meal contained in secondary metabolite from *Aspergillus flavus* (aflatoxin).

Aflatoxin can cause a wide range of acute and chronic health effects in humans. Although acute aflatoxin poisoning is rare, some aflatoxin can cause cancer in the long run [3]. Certain toxin has specific effect like affecting the immune system, liver or kidney. Since they are wide spread in nature, total elimination of mycotoxins from the food is impossible; however careful control of farming practices like selection of crop varieties is encouraged. Poor storage of foods can lead to an infection by the mould fungus *Aspergillus flavus*, releasing the toxic and highly carcinogenic substance [4].

Consequently, these mycotoxins deteriorate these foods hence reducing their market value and as well pose danger of toxicity. There are over 300 mycotoxins but the most occurring in African foods include aflatoxins, ochratoxins, fumonisins, patulin [5]. Aflatoxin B1, the most toxic compound, is usually associated with aflatoxin B2; these compounds are usually formed by both *Aspergillus flavus*and *Aspergillus parasiticus* and are ingested in feed [6] while Aflatoxin M1 and M2 are formed in milk.

Many substances support aflatoxin growth product by aflatoxigenicmoulds. Natural contamination of cereals, figs, oil seed, nuts, tobacco and a long list of other commodities is a common occurrence. Sometimes crops become contaminated with aflatoxin in the field before harvest where it is usually associated with drought stress; even more problematic in the fate of crops stored under conditions that favourmould growth. [7] suggested that in storage; usually the most important variables are the moisture content of the substrate.

Anutrients are those compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce adverse effects. Seeds of legumes and other plant sources contain in their raw state wide varieties of anutrients which are potentially toxic. It can be obtained through a diet of fresh fruits, vegetables, natural whole grains and legumes. The major anutrients includes: flavonoids, saponins, tannins etc [8]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganism *invitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. They also are effective antioxidant and show strong anticancer activities. Saponins are known to produce inhibitory effect on inflammation. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponin include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [9].

Tannins bind to proline rich protein and interfere with protein synthesis. Tannins may be classified as hydrolysable that is degradable by enzymes to yield sugar residue and phenol carboxylic acid condensed tannins. Food with high tannin contents can cause side effects such as stomach irritation, nausea, vomiting, and liver damage [10]. This study therefore, was aimed at evaluating the total aflatoxin content, anutrients level and pH profile of selected food items consumed locally in in South Eastern, Nigeria to guide producers, handlers and consumers.

MATERIALS AND METHODS:

Materials:

The plant samples used for the study include *Pentaclethramacrophylla* (oil bean), *Citrulluscolocynthis* (melon), *Arachishypogaea* (peanut butter), *Manihotesculenta* (cassava chips), *Capsicum annuum* (pepper), *Zea mays* (corn pap), and *Mondoramyristica* (African nutmeg). The samples were obtained from Agulu district, Anambra State. The samples were stored in good conditions before taking them to the laboratory for the analysis.

Methods:

Sample preparation:

The samples were processed by pulverizing (grinding) to breakdown the sample into smaller particles using industrial blender, thus increasing its surface area during the analysis. Before grinding, the surface of the compartment of the industrial blender was cleaned with cotton wool soaked in ethanol as surface sterilization and allowed to dry in a drying cabinet. The samples were then ground into powdered forms and were collected separately into sterile screw capped Duran bottles. The resulting flour samples were used for aflatoxin, anutrients and

pH analysis.

Sample processing:
The seven samples were prepared for the analysis by weighing 10g of each of each sample into a suitable plastic container with a screw cap. Then 25 mL of methanol was added to cassava chips, oil bean, corn pap and melon flours while 25mL of tween/ethanol mixture (30/70 ratio) was added to peanut butter, pepper and African nutmeg. Tween/ethanol mixture was used for the naturally colored samples. The samples were placed on a rotary shaker set at 250 rotations for 30 minutes to stir and was filtered using filter paper. This toxin extraction procedure was done according to National Agency for Food and Drug Administration and Control (NAFDAC) Standard Operating Procedure.

Determination of Aflatoxin content:
This was done according to the National Agency for Food and Drug Administration and Control (NAFDAC) Standard Operating Procedure.Multichannel pipette set at 200µl was used to pipette aflatoxin conjugate into all wells to be used. Then using multichannel pipette set at 100µl, the prepared sample was added into the well starting from well 7.Mixing was achieved using the multichannel pipette and 100µl of the mixture was transferred from each well into a new set of microwells and incubated for 15 minutes. At the end of incubation, the content was discarded and the microwells washed with de-ionized water for 5 times, and dried using an absorbent paper towel. Using multichannel pipette set at 100µl, the substrate was added into all the wells and incubated for 15 minutes (blue colour change was observed).Stock solution was added into the entire well using multichannel pipette set at 100µl (colour changed from blue to yellow). The arranged wells were placed in the ELISA reader already set for aflatoxin and the result were recorded.

3.2.2 Determination of flavonoids content:
This was done according to the method described by [11]. Ten gram (10 g) of each sample was weighed into a 250mL conical flask and 100mL of 80% aqueous methanol added and shaken for 3hours using a rotary shaker at room temperature. The extraction process was repeatedly done. The whole solution was filtered with a Whatman filter paper 1. The filtrate was transferred into pre-weighed beaker and evaporated to dryness over a water bath and cooled in desiccators to a constant weight.

% flavonoid = $\frac{W2-W1}{W3} \times \frac{100}{1}$

Where: W2 = weight of beaker + content, W1 = weight of empty beaker, W3 = weight of sample used.

Determination of saponins content:
Saponin determination was done by method of [12] as modified. Ten gram (10 g) of each sample was weighed out in conical flask and 20 % acetic acid/ethanol solution was added and allowed to stand in a water bath at 50°C for 24 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wisely to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content weighed and calculated in percentage.

% saponin content = $\frac{(weightoffilterpaper+residue)-(weightoffilterpaper)}{weightofsample} \times \frac{100}{1}$

Determination of tannin content:
The Folin-Denis spectrometric method as described by [13] was adopted. One (1) gram of each sample was weighed out and 10mL of distilled water added and agitated by shaking. This was allowed to stand for 30 minutes at room temperature with shaking at every 5 minutes interval for another 30 minutes. At the end of 30 minutes, the solution was filtered. A 2.5mL of the supernatant (extract) was added into 50ml volumetric flask and 2.5mL of standard tannic acid solution was added into a 50mL separate volumetric flask. Then, 1 mL of Folin-Denis reagent was added into each flask and 2.5mL of saturated sodium carbonate solution was added and diluted with distilled water make up to the mark in the volumetric flask. This was incubated for 90 minutes at room temperature.

Absorbance of the test sample and standard solution was read at 250nm in a Jenway model 6000 electronic spectrophotometer. Reading was taken with the reagent blank at zero (0).

% tannin= $\frac{An}{As} \times \frac{C}{1000} \times \frac{100}{W} \times \frac{Vf}{Va}$

Where; An = absorbance of first sample, As = absorbance of standard solution, C = concentration of standard solution, W = weight of sample used, Vf = total volume of extract, Va = volume of extract analyzed.

Determination of pH profile:
To determine the pH of each sample, 10g of each sample was weighed into 100mL volumetric flask and 100mL of distilled water added. The mixture was homogenized and the liquid tested using pH meter by inserting the pH electrode into the test solutions.

Reading was then recorded.

RESULTS:

Total Aflatoxin contentof the samples:

The result revealed that all the seven food items studied contained aflatoxins in varying proportions. Peanut butter had the highest concentration of aflatoxin (11.027±0.5 µg/Kg) followed by pepper (7.833 ±0.4 µg/Kg) while thelowest concentration was found in cassava chips (2.566 ±0.2µg/Kg) sample (Figure 1). The aflatoxin content of these food differed significantly at $p<0.05$.

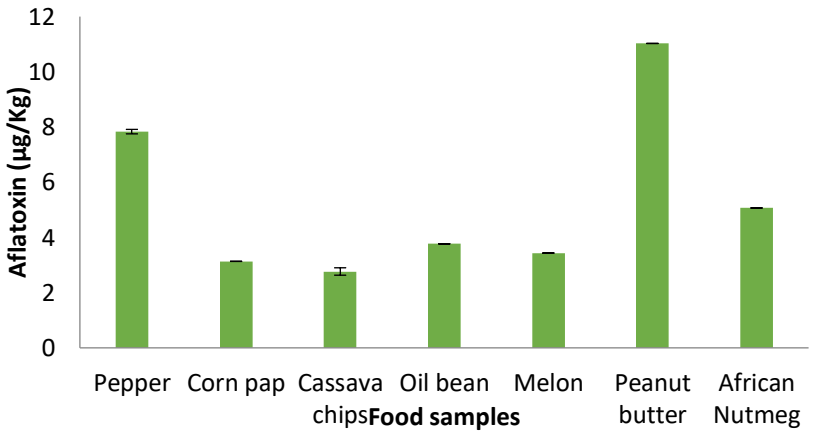

Figure 1: Total aflatoxin level in food samples

Anutrientlevels among the samples:

The result of anutrient analysis revealed the presence of flavonoid and saponins in all the food samples while tannin waspresent only in melon and African nutmeg. Pepper had the highest concentration of flavonoids, followed by peanut butter (13.2±1.5% and 7.3±0.9% respectively), while cassava chips had the lowest (1.3 ±0.3%). There were some significant variations ($p<0.05$) in the saopinin contents of the seven food samples studied.Saponin content was highest in melon (17.3±1.4%) followed by corn pap (16.9±1.3%), while peanut butter (10.1±0.8%) was the least. However, the difference in the tannin content between melon and African nutmeg, was not statistically significant ($p>0.05$) (Figure 2).

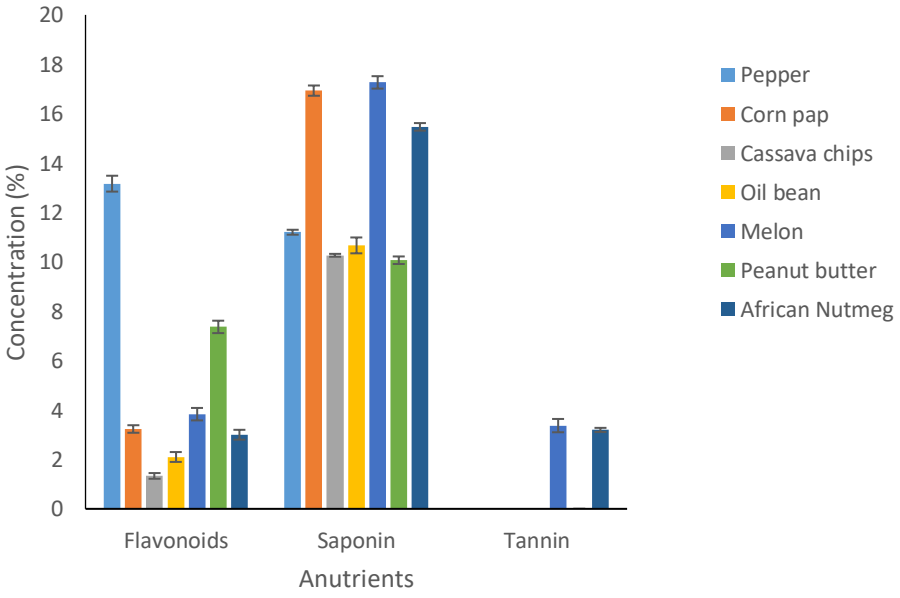

Figure 2: Anutrient(flavonoid, saponin, and tannin) contents of selected food samples

4.3 pH profile of the food samples:
The results showed that the pH of the seven samples ranged from 7.21 to 4.57 with melon recording the highest pH (7.21) followed by peanut butterwhile least was observed in corn pap (4.57) as shown in Figure 3.

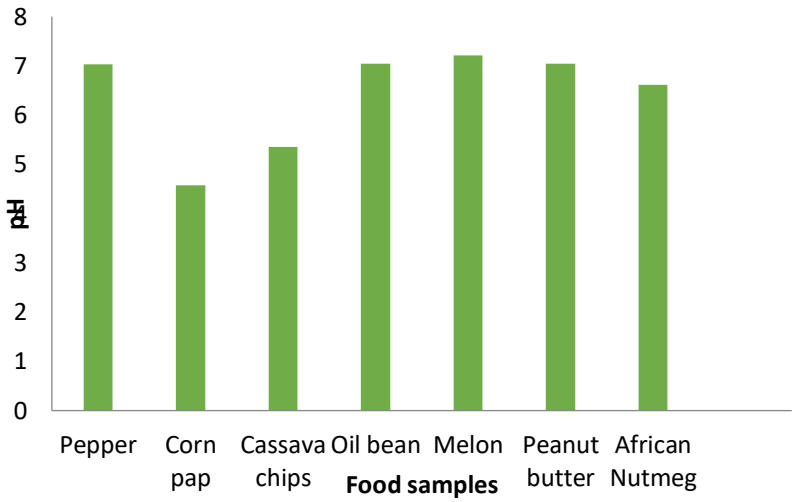

Figure 3: pH profile of the food samples

DISCUSSION:
This study showed the presence of aflatoxin in all the selected food samples. Unfortunately, aflatoxin has been associated with various diseases of human such as cancer, renal failures, liver problems, convulsion, coma and even death [14]. The study also showed that the occurrence and the amount of aflatoxin among the food samples was significantly higher in peanut butter and lowest in cassava chips sample (Figure 1). [15] explained that aflatoxins occur in crops like peanut butter because drying for storage and processing is usually delayed. This explains why peanut butter had higher aflatoxin content compared to other food samples studied. It is possible that this improper handling during storage and processing of peanut butter could make it very susceptible to fungal invasion.

Owing to their toxicity, aflatoxins should not be present in food in quantities detectable by available methods. Hence, this result points out the necessity of food inspection for contamination with fungus and presence of aflatoxins so as to keep the populace safe. This assertion is in line with [16], who stated that "in the present state of knowledge, one cannot be relied upon safe dosages of aflatoxins in foods", hence any dosage presents a serious hazard. There is also need to harvest, process and package food items in a hygienic manner to avoid contamination by fungi. This is because the occurrence of aflatoxin is influenced by certain environmental factors such as geographical locations, agricultural and agronomic practices [17]. According to [18], peanut butter is prone to quality deterioration and damage due to improper storage which could have resulted to the high affinity of aflatoxins in peanut butter and pepper than in the other selected food samples. Therefore, hermetic storage of peanut butter using Improved Crop Storage (ICS) bags is advised because they have less aflatoxin contamination and is a viable and ecologically safer storage method.

Anutrients are non-nutritive plant chemicals that have protective or disease preventive properties. Meanwhile, the results of the anutrient analysis of the foods also revealed that saponins were present in higher concentration compared to flavonoids and tannins. The flavonoid contents ranged from 13.2 % in pepper to 1.3 % in cassava chips. [19] reported that total flavonoid contents ranged from 10.28 to 15.52 mg/g in fresh peppers and that total flavonoids increases with maturation from green to red colour. Also, [20] observed that total flavonoids for green, yellow and red pepper were 7.8, 4.1 and 10.4 mg QE/100 g. This showed that ripe pepper has higher flavonoids content which may be why pepper had the highest content of flavonoids among the samples studied.

Flavonoids are naturally occurring secondary metabolite in plants and are thought to have positive effects on human health. Studies on flavonoid

derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities [21]. Flavonoids have been shown to be highly effective scavengers of the most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [22]. Also, saponins content ranged from 17.3 % in melon to 10.1 % peanut butter. Being widely distributed amongst plants, saponins have long been regarded as an anutrient which protect plants against pathogens. Therefore, it is no doubt that saponins function as potential medicinal candidates [17].

The foods were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. And from the results, melon had the highest availability of saponin than the other selected food samples.Tannins have traditionally been considered antinutritional, but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. The tannin analysis done on all the selected food samples revealed that only melon and African nutmeg showed the presence of tannin. Tannins have been found to be responsible for high immunomodulatory activity in previous studies [9]. [3] explained that tannin compounds are widely distributed in many species of plants where they play a role in protection from predation and might help in regulating plant growth.

Meanwhile, the pH profile of the food samples showed that peanut butter, melon, oil bean and pepper were alkaline while corn pap, cassava chips and African nutmeg were acidic. pH is a measure of the acidity or alkalinity of a substance. From this study, it was observed that the more acidic food samples (corn pap and cassava chips) had lower aflatoxins concentration compared to the sample which were weakly alkaline in nature. It has been stated that most fungi are slightly affected by pH over a broad range, commonly 3 to 8 [7]. Thus, this study has therefore, shown among other things that more acidic foods has reduced aflatoxin concentrations.

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