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DETERIMNATION OF AFLATOXINS IN GROUNDNUT *(ARACHIS HYPOGEA)* SOLD IN NNEWI MARKET, NNEWI NORTH L.G.A OF ANAMBRA STATE

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ABSTRACT

Aflatoxins are potent toxic, carcinogenic, mutagenic immunosuppressive agent produced as secondary metabolites by some fungi causing aflatoxicosis in humans and animals.A total of 20kg of raw and boiled bold type of groundnuts (Arachins hypoges)each were randomly collected in the dry and rainy seasons from 10 different locations at Nkwo Nnewi Market Nnewi North L. G. A, Anambra state. 25 gms of groundnuts from each location were extracted and analyzed using AOAC analytic method (Association of official analytical chemists).The results showed that aflatoxins were detected in both raw and boiled samples using high performance liquid chromatography (HPLC). The percentage positive for aflatoxin in boiled and raw samples in rainy season was 42% and 41% respectively while in dry season, the percentage positive for boiled and raw samples were 49% and 45% respectively. In dry season, the total mean value for boiled groundnut was 4.200 $\pm$ 0.362 while the raw groundnut had a total mean value of 4.110 $\pm$ 0.530. In rainy season, the total mean value for boiled groundnut was 4.940 $\pm$ 0.480 while the raw groundnut had a total mean value of 4.590 $\pm$0.545. The total content of aflatoxin was 83nglml and an average daily intake 4.15nglml during rainy season while the total content of aflatoxin during dry season was 95nglml and an average daily intake of 4.75nglml.From the result, it showed that there is a risk in consumption if taken daily, since the acceptable range for European union and food and drug agency (FAO) is from 4-20nglml.

KEYWORDS: Aflatoxins, Arachis hypogeal, Nnewi Market.

INTRODUCTION

Aflatoxins are potent toxic, carcinogenic, mutagenic immunosuppressive agent produced as secondary metabolites by the fungus; Aspergillus flavus and A parasitics, A nominus and A. niger (Sorenson et al., 1984).The two important mycotoxins are aflatoxin, produced by Aspergillus flavus and A. parasiticus; and fumonism produced by fusarium verticilliodes. Both are commonly found in legumes and cereal crops. Aflatoxins have been associated with various diseases such as aflatoxicosis, in livestock like cattle, horses, rabbit and non- human primates, domestic animals and humans throughout the world.

The aflatoxin problem was first recognized in 1960, when there was severe outbreak of a disease known as "turkey x disease" in U. K, in which over 100,000 turkey poults died. The cause of the disease was shown to be due to toxins in peanut meal infected with Aspergillus flavus and the toxins were named Aflatoxins (Reddy *et al*., 2002). There are 18 known aflatoxins but most of these are metabolites formed endogenously in animals administered by one major toxins aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2. *A. flavus* produces one of the four types of aflatoxin.

Groundnut or peanut (Arachis hypogea) is a specie in legume or "bean family" (fabaceae). It is an annual herbaceous plant growing 30 -50cm (0.98-1.6ft) tall. The leaves are opposite, pinnate with four leaflets. The flowers are typical pea flower in shape, 2-4cm across, yellow with reddish veining. Peanuts grow best in light, sandy loamy soil. They require five months of warm weather and an annual rainfall of 500 -1000 cm or equivalent in irrigation water.

Poor storage, high crop densities and weed competition have all been associated with increased mold growth and

toxin production. Poor storage can lead to an infection by the mould fungus *Aspergillus flavus*, releasing the toxic and highly carcinogenic substance aflatoxin. Preharvest contamination of peanut is favoured by warm temperature and high humidity while aflatoxin contamination of corn is favoured by high temperature, prolonged drought condition and high insect activity. Aflatoxin producing molds exists throughout the peanut growing areas and may produce aflatoxins in peanuts when conditions are favourable to fungal growth (Seijo *et al*., 2007). Groundnuts are mostly contaminated with Aflatoxin B_1 and B_2, less often with aflatoxin G_1 and G_2; so it is important to have analytical values that represent the total aflatoxin content (Ehrlich *et al*., 1984).

Evidence of acute aflatoxicosis in humans have been reported from many parts of the world, namely Taiwan, China, Uganda, India, Africa and many others. The syndrome is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma and death with cerebral edema and fatty involvement of the liver, kidney and heart. The condition is most common in poorly developed countries where there are insufficient controls on the presence of aflatoxin in food.This paper attempts to review aflatoxin's research on groundnuts and consequently its effects on human health.

The aim of this research is to detect the presence and level of aflatoxin in groundnut sold in Nnewi market.

MATERIALS AND METHOD

Groundnut (Bold type) comprises of boiled and fresh (raw) – 20 kg. HPLC (High performance liquid chromatography).

Sample collection

The groundnut (Arachins hypoges) was collected from Nkwo Nnewi Market while the analysis was done in central research unit. Nafdac Lagos.

Method

200g each of the randomly selected bold types of groundnut were collected and pooled to obtain an aggregate of 20kg which is in line with codex Alimentarius commission 1999 that currently established a sampling plan that requires 20kg sample for analysis.

Extraction

25gs each from the boiled and raw groundnut was extracted and analyzed for aflatoxin using AOAC analytical method (991. 3) respectively. Ten samples of both groundnuts were tested for aflatoxins.

25gms on each sample were ground and weighed. It was blended with salt and methanol/ water mixture (80:20). The mixture is shaken and left for 30 mins to 1 hour, after which it is filtered.

Procedure (using HPLC)

100 ul of the extract was added to 400ul of water and mixed. Using the lml per min flow rate of the mobile phase, inject 20ul of the filtrate of standard and test extracts. Record digital readout after 1 min. calculation curves were prepared using standard solutions of aflatoxins AfB_1, B_2, G_1 and G_2. (AfG_1= 1. 965, AfG_2 = 1. 404. AfB_1, = - 9. 82, AfB_2 = - 2.807)nglml of each aflatoxin. Quantification limits for the aflatoxins, AfB_1, AfB_2, AfG_1, AfG_2 was determined by the amount of toxin that could generate a chromatographic peak three times over the base line standard.

RESULTS

Table 1, shows the result of aflatoxins detected in boiled groundnuts during rainy season. The total aflatoxin content for boiled groundnut was 42nglml, an average of 4.2nglml intake. AfG_1 had the highest aflatoxin content – 2.0nglml while AfB_1, the lowest content with 0.2nglml.

Table II: shows the result of aflatoxin detected in raw groundnuts during rainy season.The total aflatoxin content for raw groundnut was 41nglml, an average of 4.1nglml intake.AfG_1 had the highest aflatoxin content of 2.2nglml while AfB_1 and AfB_2 had the lowest level of aflatoxin each at 0.4glml.

Table III, shows the result of aflatoxin detected in boiled groundnut during dry season. AfG_1 had the highest aflatoxin content of 2.2nglml while AfB_1 and AfB_2 had the lowest level of 0.4nglml.

Table IV: shows the result of aflatoxin detected in raw groundnut during dry season. The total aflatoxin content was 45.9nglml; an average of 4.5nglml .AfG_1 had the highest aflatoxin content of 2.2nglml while AfB_1 and AfB_2 had the lowest level of 0.4nglml.

Table V: shows the mean value, T-test, P-value and the inference of both boiled and raw groundnut during rainy season. The total mean value of boiled groundnut had 4.200 ± 0.362 the raw groundnut had 4.110 ± 0.530, T-test of 0.443 and P- value is significant when $P < 0.05$.

Table VI: shows the mean value, T-test, P- value and inference of both boiled and raw groundnut during dry season.

The total mean value of boiled groundnut had 4.940 ± 0.480 while the raw groundnut had 4.590 ± 0.545, T-test of 1.522, p-value of 0.064 which is not significant.

The mean value of AfB_2 had 1.040 ± 0.196 in boiled and 0.800 ± 0.288 in raw groundnut; with a T-test of 2.189, P- value of 0.015 which is significant in dry season while AfG_2, AfB_1 and AfB_2 are significant in rainy season with p-value of 0.000, 0.002 and 0.002 respectively.

Table 1: Showed the result of aflatoxin in boiled groundnut during rainy season Result for boiled groundnut (during Rainy season).

sample	AfB_1	AfB_2	AfG_1	AfG_2	Total
1	0.5	1.0	2.0	1.4	4.9
2	0.2	0.7	1.6	1.1	3.6
3	0.3	0.9	1.7	1.2	4.1
4	0.4	0.7	1.8	1.3	4.2
5	0.2	0.8	1.9	1.2	4.1
6	0.5	0.8	1.5	1.2	4.0
7	0.3	0.9	1.5	1.4	4.1
8	0.4	1.0	1.6	1.2	4.2
9	0.5	1.0	2.0	1.2	4.7
10	0.5	1.0	1.5	1.1	4.1

42nglml

Table 2: showed the result of aflatoxin in raw groundnut during dry season Result for raw groundnut (nglml) during dry season.

	AfB_1	AfB_2	AfG_1	AfG_2	Total
1	0.5	0.5	2.0	1.4	4.4
2	0.6	0.5	1.1	1.4	3.6
3	0.8	0.6	1.2	1.2	3.8
4	0.7	0.4	1.5	1.3	3.9
5	0.4	0.7	2.2	1.6	4.9
6	0.5	0.4	1.1	1.4	3.4
7	0.6	0.5	2.0	1.4	4.5
8	0.5	0.5	1.1	1.4	3.5
9	0.6	0.5	2.1	1.4	4.6
10	0.5	0.5	2.0	1.5	4.5

41nglml

Table 3: showed the result of aflatoxin in boiled groundnut during rainy season Results for boiled groundnut (rainy season).

	AfB_1	AfB_2	AfG_1	AfG_2	Total
1	0.5	1.5	2.2	1.6	5.8
2	0.3	1.0	1.8	1.5	4.6
3	0.5	1.0	1.8	1.4	4.7
4	0.5	0.8	2.0	1.8	5.1
5	0.3	0.8	2.0	1.8	4.9
6	0.7	0.9	1.5	1.5	4.6
7	0.4	1.0	1.6	1.3	4.5
8	0.5	1.1	1.6	1.3	4.5
9	1.0	1.1	2.0	1.1	5.2
10	1.0	1.2	2.5	1.0	5.7
					49.4
					Average= 4.94nglml

Table 4: showed the result of aflatoxin in raw groundnut during rainy season Results for raw groundnut (rainy season).

	AfB_1	AfB_2	AfG_1	AfG_2	Total
1	0.6	0.6	2.2	1.5	5.9
2	0.8	0.6	1.4	1.5	4.3
3	0.7	0.4	1.5	1.4	4.0
4	0.5	0.6	2.2	1.6	4.9
5	0.4	0.8	1.2	1.5	3.9
\6	0.6	0.5	1.1	1.6	3.8
7	0.5	1.2	1.6	1.3	4.6
8	0.4	1.2	1.8	1.4	4.8
9	1.0	1.1	2.1	1.0	5.2
10	1.0	1.0	2.0	1.5	5.5
					45.9
					Average= 4.59nglml

Table 5: showed the mean value, T-test P-value and inference of both boiled and raw groundnuts during dry season.

	Boiled groundnut mean ± SD	Raw groundnut mean ± SD	T- test	P – value	Inference
AfB_1	0.380±0.123	0.570±0.116	3.556	0.002	Significant
AfB_2	0.880±0.123	0.510±0.472	7.753	0.000	Significant
AfG_1	1.710±0.203	1.630±0.472	0.493	0.628	Not Significant
AfG_2	1.230±0.106	1.400±0.105	3.597	0.002	Significant
Total	4.200±0.362	4.110±0.530	0.443	0.663	Not Significant

$P < 0.05$ (Significant)

Table 6: showed the mean value, T-test, P-value inference of both boiled and raw groundnuts during rainy season.

	Boiled groundnut mean ± SD	Raw groundnut mean ± SD	T- test	P – value	Inference
AfB_1	0.570±0.241	0.650±0.211	0.790	0.215	Not Significant
AfB_2	1.040±0.196	0.800±0.288	2.189	0.015	Significant
AfG_1	1.900±0.289	1.710±0.388	1.242	0.108	Not Significant
AfG_2	1.430±0.253	1.430±0.168	0.000	0.500	Not Significant
Total	4.940±0.480	4.590±0.545	1.522	0.064	Not Significant

DISCUSSION

From the result obtained, it is obvious that the presence of aflatoxins B_1, B_2, G_1, and G_2 were detected in both boiled and raw groundnut in both seasons (Dry and rainy season).

In D' mell (1997) report, geographical distribution on strains varies*; A Flavus* produces aflatoxin B_1 B_2, and G_2 worldwide while *A Parasiticus* produces B_1, B_2, G_1 and G_2 in Africa and America, so the samples which were randomly collected had all the types of aflatoxin therefore *Aspergillus flavus* and *A. parasiticus* might have been the cause of contamination of these *groundnut* samples. Raw samples analyzed by Carlos *et al.* (2009), in Brazil also had all the aflatoxin strains B_1, B_2, G_1 and G_2.

In turkey, 20 samples of peanut from one company were analyzed by Yentur *et al.* (2009) and all samples contained aflatoxin with total aflatoxins Af (B_1 B_2, G_1) ranging from 8.16 to 75. 74ugkg.

Also Carlos *et al.* (2009), had total mean value and standard deviation of aflatoxins as follows; AfB_1 = 6. 02 $\pm$13.58, AfB_2 = 0.74 $\pm$1.34, AfG_1 = 4.39$\pm$1.030, AfG_2 = 1.73 $\pm$ 4.13 using high performance liquid chromatography as compared to this present study which had AfB_1 = 0.380 $\pm$ 0.123, AfB_2 0.880 $\pm$0.123, AfG_1 = 1.710 $\pm$ 0.203, AfG_2 = 1.203 $\pm$ 0.106 for boiled groundnuts and AfB_1 = 0.570 $\pm$ 0.116, AfB_2 =0.510 $\pm$ 0.472, AfG_1 = 1.630 $\pm$ 0.472. AfG_2 = 1.400 $\pm$0.105 for raw groundnut.

In rainy season, AfB_1, AfB_2 and AfG_2 had P-values of 0.002, 0.000,0.002 which were of significant since the P-values of less than 0.05 is of significant (<0.05) while in dry season, only AfB_2 had a significant P- value of 0.015.

In indonesia, 24 samples of peanuts were collected and analyzed by Okky *et al*. (2007) in both wet and dry seasons, the aflatoxin B_1 levels collected and analyzed were higher than the dry season. Percentage positive for aflatoxin in wet season in retail samples and those collected from collectors and farmers were 4%, 17% and 33% respectively.

During the dry season, around 42% and 74% peanut samples collected from collectors and farmers were contaminated with more than (4-20nglml) or 15ppb.

In this study, the percentage positive for aflatoxin in groundnut sampled in dry season was 42% and 41% for both boiled and raw groundnuts respectively. While in rainy season, the percentage positive was 49% and 45%. According to European Union and FAO, 2004, the proposed maximum units for aflatoxins in foods and feeds commonly range from 4-20nglml.

My study shows that it is not safe for consumption if taken daily since the daily intake is 4.1nglml in rainy season and 4.8nglml in dry season.

CONCLUSION

In conclusion, the aflatoxin level detected in the groundnut sampled and analyzed from Nkwo Nnewi market which amounts to 4.1-4.8nglml daily intake indicates a significant risk in consumption. Therefore, awareness should be created for proper storage and preservation, through mobilization in the market in order to prevent contamination and reduce the effect of aflatoxicosis that leads to liver cirrhosis, hepatitis and liver cancer.

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