

Mycotoxin Levels in Groundnut Paste in Navrongo in the Upper East Region of Ghana

Martin Agyei

Ghana Education Service, Diaso Senior High School, Post Office Box DW 13 Diaso, Central Region, Ghana

Patrick Frimpong

Ghana Education Service, Adu Gyamfi Senior High School, Post Office Box 17, Jamasi-Ashanti, Ghana

Abstract

Aflatoxin, is a toxin from a naturally occurring mould, it is a Group 1 carcinogen proven to cause cancer in humans. Aflatoxin can also cause economic losses in livestock due to disease or reduced efficiency of production. Groundnuts/Peanuts contribute significantly to food security in the Northern Ghana (precisely Navrongo) due to their high nutritional value and cash crop potential. However, the crop is highly susceptible to aflatoxin and contamination. Yet little information is available on the extent of contamination in the region. This study explores the level and extent of contamination of peanut paste by mycotoxins (aflatoxins) in Navrongo market in the Upper East Region in Northern Ghana. A cross sectional study was used to conduct research and a survey of groundnut paste was collected from the Navrongo market in the Upper East Region of the Northern Ghana. The sample size was 10 different groundnut paste from ten groundnut paste sellers who were selected using simple random sampling. Ten samples were taken from these sellers, labelled from A to J and sent to the laboratory for analysis. Samples were prepared by mixing with extraction solution, blending and filtering. The extract was then applied to the AflaTest columns bound with specific antibodies to aflatoxin. At this stage, the aflatoxin binds to the antibody on the column. The column is then washed with water to rid the immune affinity column of impurities. By passing methanol through the column, the aflatoxin is removed from the antibody. This methanol solution was then measured in a fluorometer. Aflatoxin levels of each sample collected were determined by Affinity Chromatography (VICAM) method. Extraction of aflatoxin in the samples was done on the affinity chromatographer, identification of the levels of aflatoxin B1 in the samples were determined with a fluorometer (VICAM).

Keywords: Mycotoxins, Aflatoxin, Groundnut Paste, Extraction, Upper East, Ghana

1.0 Introduction

Mycotoxins are poisonous substances (toxins) produced by fungus; these secondary metabolites produced by fungi are capable of causing disease and death in humans and other animals. Some may have a survival advantage by virtue of their toxicity to competing organisms in their micro environment. The biological functions of others are unclear but many have significant biological activity and several are toxic to mammals (humans). They therefore have significant public health and economic implications. It is estimated that 25% of the world's annual food crops are contaminated by Mycotoxins. (FAO/WHO Geneva, 200)

Some Mycotoxins are of interest to pharmacologist and toxicologist because they have served as research tools to study cell function and to identify various types of neurotransmitters and blocking agents (Richard, 2001). This project will focus on the mycotoxin (Aflatoxin) levels in consumable peanut paste/butter in Navrongo (northern Ghana), the various ways of extracting the toxic substance from a sample of groundnut paste, and the possible remedies and ways of averting these dangers to human health.

1.1 Background of the study

Mycotoxins are poisons produced by fungal growth on cereals, nuts, fruits, and vegetables. More than 100 species of fungi produce this toxin. Under certain conditions, these fungi can infect and grow in various crops, including peanuts and corn, contaminating them with mycotoxins. Commonly found in corn, groundnut paste /peanut paste and tree nuts, the toxin also can be transmitted to humans through the milk, meat or eggs of animals fed with contaminated grains. (Richard, 2001)

The most common mycotoxin and the one of interest to this project is **aflatoxin B1** produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin contamination of peanut Paste is a food safety concern and major economic burden for the peanut industry. Aflatoxin contamination occurs when seeds are colonized by the mould, *Aspergillus flavus*, under late-season conditions of drought and high temperature- *conditions which are prevalent in the Navrongo area*. Because mycotoxin contamination threatens the safety of food and feed, extensive monitoring must be done to ensure that mycotoxins are below levels safe for consumption. Aflatoxin has been found to be the most potent carcinogen or potentially cancer-causing agents yet discovered (Mintah, *et al.*, 1978)

Other Mycotoxins include trichothecenes and zearalenone compounds known to injure the intestines,

bone marrow, and lymph nodes as well as spleen and thymus (organ of immune system; an organ located at the base of the neck that is involved in the development of cells of the immune system, particularly T cells). They are produced by species of fusarium that grow on grain, straw or heavy stored while damp (Pitt, 2000).

1.2 Problem statement/Justification

Mycotoxins contaminate a wide variety of food as a result of fungal infection in crops, during growth or in storage. Aflatoxins produced by *Aspergillus flavus* are known to have acute and chronic toxic effects. The most toxic is aflatoxinB₁ (AFB₁) that is classified as human carcinogen. (Richard, 2001). Peanuts are sold as raw kernels, roasted nuts, or processed into peanut butter. The nuts are rich in protein (Mehan *et al.*, 1991) and are an ideal alternative to fish, which is less expensive in northern Ghana, thereby playing a significant role in food security. Aflatoxins occur as aflatoxinB₁ (AFB₁), AflatoxinB₂ (AFB₂), AflatoxinG₁ (AFG₁), AflatoxinG₂ (AFG₂) and they are found in cereal products, cocoa products, nut products such as peanut, spices, wine and beer, dry fruits and baby foods, animal feeds etc. Aflatoxin M₁ (AFM₁) and M₂ (AFM₂) are found in milk. (Eaton, and Gallagher, 1994).

In an attempt to find the presence of aflatoxin in the food products in the Navrongo area, this project researched into groundnuts paste (one of the staple foods in the research area) to establish the presence or absence of aflatoxin in it. Aside the effects of mycotoxins being fatal, it is responsible for considerable morbidity and causing social stigma among men women and children. (WHO 2006). In 2009 a company called “Jesu Aka”-A Ghanaian based company in the United Kingdom supplied peanut paste to the UK market, upon analysis of the food, the levels of mycotoxins discovered in the paste was (35ppb), 35ug/ml so high that it resulted in serious sickness to most Children who consumed the paste as spread on bread. The authorities on discovering this banned the product from the UK market. (www.ehso.com) Assessed on 5th December 2011. It is known that cancer caused by mycotoxins or Aflatoxins can be lethal that deaths can occur in humans consuming an estimated 6 mg/day of aflatoxin B₁. (Richard, 2001).

1.3.0 Objectives

1.3.1 General Objective

To identify the mycotoxin levels of groundnut paste in Navrongo area.

1.3.2 Specific Objectives

- To extract mycotoxins (aflatoxin) in groundnut paste
- To study the morphological characterisation of aflatoxins
- To determine the level of aflatoxin B₁ in each sample collected

2.0 LITERATURE REVIEW

Peanut (*Arachis hypogaea* L.) or groundnut, is a four-foliolate legume of the family *Fabaceae*. Native to South America, peanut is produced in China, India, the United States of America and many Sub-Saharan African countries. Developing countries account for 92 per cent of total global groundnut production (Talawar *et al.*, 2005; ICRISAT, 2005). The four common market types are: i) Spanish-small kernels with reddish-brown skins, ii) Runner-have a consistent medium size-, iii) Virginia-have an extra large kernel size- and iv) Valencia-have three or more kernels to a shell and are bright red- (Edinformatics, 2005).

The local varieties of peanuts in the research area, Navrongo in the upper east region are: manipintar locally called ‘sunupupuru’, F-mix (not common), and Chinese locally called ‘sunugua’ (MOFA U/E, 2012). Peanut paste is high in protein (26 to 39 per cent), fat (47 to 59 per cent) and carbohydrates (11 per cent) (Nelson and Carlos, 1995; Atasié, Akinhanmi and Ojiodu, 2009). It contains several minerals, including Na (42.0 mg/100g), K (705.11 mg/100g), Mg (3.98 mg/100g), Ca (2.28 mg/100g), Fe (6.97 mg/100g), Zn (3.2 mg/100g) and P (10.55 mg/100g) (Atasié *et al.*, 2009), as well as vitamins E, K and B (Technical Advisory Committee, 1997). Due to its high nutritional value, it has several uses such as weaning and therapeutic food, in confectionery, and as an animal feed.

Most commercially available peanuts pastes are processed by small and micro-enterprises, a sector that contributes approximately 18 per cent of Ghana’s Gross Domestic Product (Mitullah, 2003).

Mycotoxins are produced by certain fungi (e.g., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.) that grow on human food and animal feed ingredients such as corn, sorghum, wheat, barley, peanuts, and other legumes and oilseeds. Five broad groups of mycotoxins—aflatoxin, vomitoxin, ochratoxin A, fumonisins, and zearalenone—are commonly found in food and feed grains. (Ames, 2003). Among mycotoxins, probably the most widely recognized risk comes from aflatoxins. Aflatoxins are extremely potent carcinogenic and mutagenic substances that first came into the public spotlight—and were formally identified—in the early 1960s following the deaths of more than 100,000 young turkeys on a poultry farm in England. The so called Turkey X disease was eventually tied to high levels of aflatoxin in Brazilian peanut meal imported as a feed ingredient. (Orriss, and Gregory, 1997).

Aflatoxin contamination is most common in African, Asian, and South American countries with warm and humid climates, but also occurs in temperate areas of North America and Europe, these five groups of mycotoxins all pose health concerns and are subject to Sanitary and Phyto sanitary (SPS) or other regulatory measures, which states that measures which conform to Codex standards, guidelines, or other recommendations are science-based, appropriate, and non-discriminatory” (Park et al., 1999). Guerzoni and Maria, tried to find out the exact locations where the grain under study (groundnut) get contaminated before reaching the final consumption, they found out that even though groundnuts are roasted to higher temperatures before grounded to pastes, they still contain some mould contaminations (Guerzoni and Maria 1999).

Below is the preview of their study which led to the formulation of the article above.

The fungi that produce mycotoxins can emerge either in the field (in soil, decaying vegetation, and grains undergoing microbiological deterioration) or during post harvest transportation or storage. A further concern is that the absence of visible mold does not guarantee the grain is free from mycotoxin and cooking or processing the food product does not necessarily rid it of mycotoxin contamination. For example, mold contaminated with aflatoxins have been isolated in processed food products such as bread, macaroni, cooked meat, cheese, and flour (Guerzoni, 1999).

The major method used for reducing aflatoxin levels in peanuts is colour sorting. In this procedure, nuts are inspected individually by electronic or laser sorting systems and discoloured nuts removed. The rationale for aflatoxin reduction by colour sorting is that the growth of a fungus in peanut results in discoloration, so removal of discoloured nuts sorts out those containing aflatoxins as well. In the United States and Australia, it is standard commercial practice that every individual shelled peanut entering commercial stream has been colour sorted. If the colour sorting process is ineffective, as can occur when severe drought stress causes peanuts to commence drying in the soil before harvest (Mitt, 2004). This researcher worked on **reduction** of mycotoxins/aflatoxins levels in groundnut pastes. He failed to use the method of tin layer chromatography (TLC) to find the levels of mycotoxins/aflatoxins in the (groundnut paste) samples.

Methods of analysis of mycotoxins/aflatoxin in food generally requires a first step involving toxin extraction from the matrix with an adequate extraction of solvent, a clean up step intended to eliminate any possible interference from the extract and finally detection/determination of toxin by suitable analytical instrument or techniques, it involves the use of immuno affinity columns (IAC), solid phase extraction (SPE) or multifunctional clean-up columns. Different chromatographically methods are commonly used for quantitative determination of mycotoxin (aflatoxin), including high performance liquid chromatography (HPLC) coupled with UV fluorescence or mass spectrometry (MS) detector and thin layer chromatography (TLC). (Afriyie, *et al.*, 2005).

In mycotoxin research, one of the most pressing practical aspects is the need for rapid screening methods for its detection. Rapid detection methods are needed at elevators and mills where equipment must be simple and inspection must be completed in a matter of minutes.

Two rapid methods for aflatoxin detection now exist. The first is presumptive and is based on the occurrence of a characteristic bright-greenish glowing yellow fluorescence observed in broken kernels under an ultraviolet lamp at 365 nm. This 'firefly glow' is not aflatoxin, but is usually present in kernels of corn containing aflatoxin; the second method is the use of the minicolumn (VICAM) techniques cited officially for grains. (Shotwell *et al.*, 1972; Heseltine & Shotwell, 1972)

Mycotoxin contamination is particularly prevalent in developing countries in tropical areas such as in South Asia and Africa. High-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) are two of the most widely used methods for the detection and quantification of mycotoxins in developing countries. A number of Enzyme Linked Immuno sorbent Assay (ELISA) kits are now commercially available for the detection of aflatoxins, deoxynivalenol, fumonisins, ochratoxin, and zearalenone (Schmale and Munkvold, 2009). These methods, however, are time-consuming, difficult to use and require enough laboratory facilities. The lack of precise chemical assays is the reason we have little data on the natural occurrence of mycotoxins in cereals. (Heseltine, 1974).

Although various prevention strategies have been implemented in many countries, they were not enough to manage the problem efficiently in the developing world. In the recent years, many dietary strategies involving microorganisms have been under investigation. Many species of bacteria and fungi have been shown to enzymatically degrade mycotoxins (Ciegler *et al.*, 1966; Bata and Lasztity, 1999). However, the question still remains on the toxicity of the degradation products.

All the researchers above worked on trying to find just the presence of mycotoxins/aflatoxins in cereals and grains without particularly focusing on the specific sample and its associated levels of toxins present. The researcher will focused on a specific number of groundnut paste samples and their related levels of aflatoxins present.

As a cereal crop, peanut or groundnut is one of the most important food and feed commodities. However, mycotoxin contamination of it represents a widespread problem. In fact, groundnut can be easily

contaminated by toxigenic mould such as *Aspergillus* and *Fusarium* species that are important either as plant pathogens in the field or as the source of mycotoxin contaminants during storage. Several issues are associated with grain moulds and their secondary metabolites in maize, i.e. mycotoxins, including lowered grain quality, adverse effects on human health, and on animal health and reproduction (Fink, 1999; Hussein *et al.*, 2001).

Although numerous toxic fungal metabolites can be found in groundnuts, attention has focused on the few mycotoxins that occur with greater frequency such as aflatoxins that are a group of secondary metabolites produced by the *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins B1, B2, G1, and G2 are a principal public health concern because of their pivotal role in the occurrence of primary liver Cancer. Since 1993 (Magan *et al.*, 2000). Establishing tolerance levels of aflatoxin in peanut products - and indeed in other crop commodities - has remained contentious resulting in different standards for the same commodity. Efforts have been made to harmonise standards, but no common standards have been agreed upon, partly due to competing trade interests (Egmond, 2000; Kendra and Dyer, 2007). For populations that rely on peanut as a source of food, tolerance levels for aflatoxin have a direct impact on food availability and safety. Stricter standards are unlikely to improve health significantly as local produce is not necessarily subjected to inspection (Wu, 2004).

Aflatoxin B1 has been classified by the International Agency for Research on Cancer as carcinogenic to humans (group 1) (IARC, 1993). The European Commission fixed maximum levels for aflatoxin B1 (5.0 µg.kg⁻¹) and the total (B1, B2, G1, G2) aflatoxins (10.0 µg.kg⁻¹) in “groundnut to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs” (Commission Regulation (EC) No 1881/2006). Rapid methods for the determination of mycotoxins in cereals are highly needed in order to prevent the entry of mycotoxins into the food chain and thereby mitigate the human and animal risk. (Keshri *et al.*, 2000; Magan *et al.*, 2000; Falasconi *et al.*, 2005; Paolesse *et al.*, 2006; Presicce *et al.*, 2006; Sahgal *et al.*, 2007).

The committee on Food Additives and Contaminants during their thirty fourth summits also worked on mycotoxin levels, but their work was mainly on the effects of the presence of these toxins in food having on the human body. (Codex Committee on Food Additives and Contaminants 2002). Consuming food products that contain high levels of certain mycotoxins can cause the rapid onset of mycotoxicosis, a severe illness characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and in rare cases, death. Although lethal cases are uncommon, acute illnesses from mycotoxins, particularly aflatoxins (aflatoxicosis), have been reported from many parts of the world, usually in developing countries. Some notable outbreaks include the deaths of three (3) Taiwanese in 1967, and the deaths of more than 100 people in Northwest India in 1974. Both outbreaks were attributed to aflatoxin contamination, of rice in Taiwan and corn, wheat and peanut (groundnut) in India. (Thirty fourth Session Codex Committee on Food Additives and Contaminants, Mar. 2002).

Thirteen countries are known to have no specific regulations, and no data are available for about 50 countries on mycotoxins, many of them in Africa (Van, 1999). In 1996, for example, 48 countries had established tolerance levels for total aflatoxins in food—up from 30 in 1987—with standards ranging from 0 parts per billion (ppb) to 50 ppb Survey data by the FAO also reveal that the number of countries adopting mycotoxin regulations grew significantly from the mid-1980s to mid-1990s, and that the range of tolerance levels vary widely (Van, 1999).

The writers below also tried to answer the question 'How widespread is the natural occurrence of mycotoxins in foods and feeds?' To answer this question, one may approach the question from several directions, most of which are only circumstantial. First, fungi can be isolated from the substrate in pure culture, grown on a suitable medium and tested in an experimental animal for toxicity. If the animal responds in an adverse fashion, one can say that the strain isolated from this substrate is toxigenic. For example, (Scott, 1965) isolated 228 mold strains from cereals and legumes, grew these on maize meal, tested them by feeding the molded meal to ducklings and recorded toxicity. He found that 46 strains representing 22 different species caused death in 14 days. (Scott, 1965).

A second approach is to select from a culture collection moulds found commonly in a commodity and screen each isolate by growing it in pure culture and then assaying it for toxin formation. (Heseltine *et al.*, 1972) took 44 representative strains representing all species belonging to the *Aspergillus ochraceus* series) grew them separately on wheat and corn and assayed them for ochratoxin. All but two of the nine species in *ochraceus* series produced detectable amounts of ochratoxin. (Hesseltine *et al.*, 1972)

A third approach in obtaining an answer to the question how prevalent is naturally occurring mycotoxins in foodstuffs, is to find outbreaks in animals of disease suspected of being caused by mycotoxins and to isolate the predominant moulds from the feed, grow them in culture on the same substrate and test in an experimental animal. One then relates the symptoms of the test animal with those observed in the original illness. (Ciegler, 1969) acquired a mouldy commercial feed suspected of having caused deaths in dairy cows and from it isolated the predominant mold, *Penicillium palitans*. When this strain was grown on sterile cracked corn, this molded grain caused ataxia, convulsions and death in mice. In each approach, the findings are only

circumstantial. The direct method is to examine suspected food or feed by chemical and biological means and determine whether a specific mycotoxin is actually present. (Ciegler, 1969)

Contamination of peanut by aflatoxin producing fungi and subsequent toxin production can occur at pre- and post- harvest (Dorner, 2008; Holmes *et al.*, 2008). Several factors therefore influence fungal colonization and toxin production. Aflatoxin contamination of groundnut is widespread where the crop is grown under rain fed conditions (Reddy *et al.*, 2003). End-season drought stress and elevated soil temperatures common in Sub-Saharan Africa promote aflatoxin contamination (Bankole *et al.*, 2006; Rachaputi *et al.*, 2002). Attack of peanut pods by pests and diseases contribute to aflatoxin contamination (Mehan *et al.*, 1991; Waliyar *et al.*, 2003). Some varieties are less susceptible than other varieties (Kasno, 2004; Reddy *et al.*, 2003). Poor seed storage, mechanical damage during harvesting, poor or inadequate drying, and poor transportation lead to conditions conducive to contamination (Waliyar *et al.*, 2005; Jones and Duncan, 1981; Bilgrami and Choudhary, 1990).

3.0 RESEARCH METHODOLOGY

3.1 Study area

The research was carried out in Navrongo in the Kasena Nankana District in the Upper East Region, Ghana. It is bordered by the republic of Burkina Faso to the North and Bolgatanga to the East. To the west are Builsa and Sissala Districts and to the south is west mamprusi district. The district capital is Navrongo. The district occupies a land mass of about 1674km², the topography is low lying with an average height of 100m above sea level. The terrain is undulating with isolated hills dotting the landscape. The vegetation of the district is of the Sudan and savannah type with grassland separating deciduous trees. (District Assembly, Navrongo, U/E, 2012).

3.2 Study design

This research adopted a cross sectional study approach involving the collection of groundnut paste samples from *sellers* for laboratory analysis. Cross-sectional research study is based on observations that take place in different groups at one time.

3.3 Sampling Technique

Simple random sampling was used to select samples of groundnut paste from the market sellers in order to make the sample very representative of the study population. The study was carried out among *groundnut paste* sellers in Navrongo market between the period of March and April 2012.

3.4 Sampling Technique

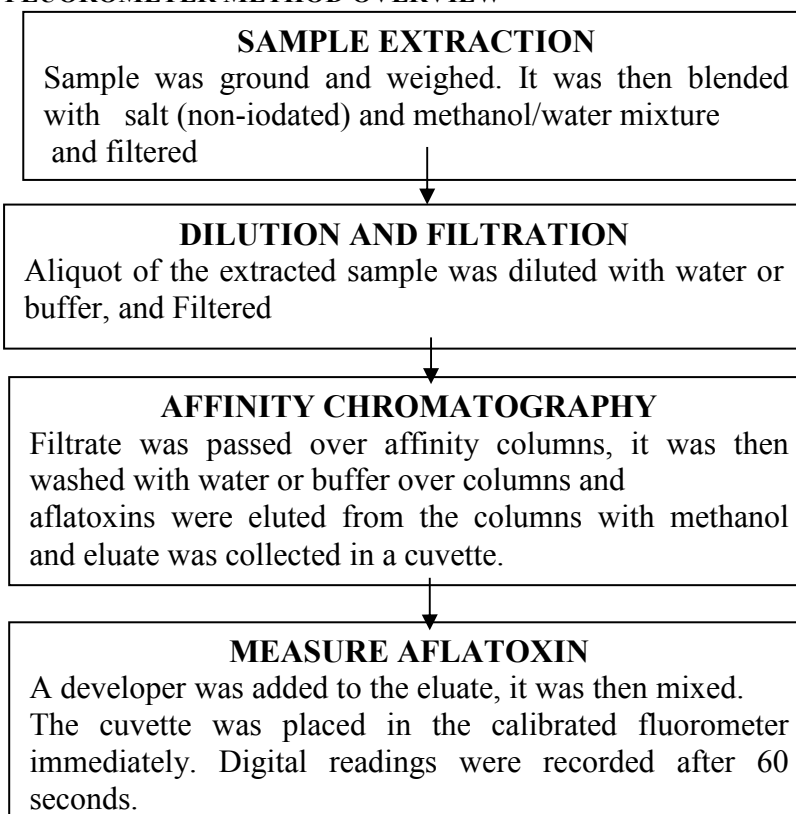
The sample size was 10. Therefore, 10 different groundnut paste from ten groundnut paste sellers who were selected using simple random sampling. Ten samples were taken from these sellers, labelled from A to J and sent to the laboratory for analysis.

TABLE 3. 1: Samples for the study and the places of collection

SAMPLES	LOCATION
A	At the main entrance to the Navrongo market
B	At the back of the MTN office in the market
C	Within the yam sellers place
D	opposite the main abattoir in Navrongo market
E	In front of the tomato sellers in the market
F	Opposite the vulcanizing/fitting shop
G	Opposite the MELCOM shop
H	In front of the mobile phone repairers shop
I	Within the vegetables sellers
J	Towards the fishmongers place

Before samples were taken, the ten well labelled containers were cleaned with cotton wool containing 70% alcohol. This was to ensure that bottles that have been already contaminated with any fungi species would be sterilized. The groundnut paste samples taken at various sample points/places were taken to the laboratory for analysis.

3.5 AFLATEST FLUOROMETER METHOD OVERVIEW



Standard aflatest fluorometer method overview

3.6 PREPARATION OF FILTRATION STEPS

3.7 Fluted Filter

The first filtration step is a simple gravity filtration through fluted filter paper to separate the sample extract solution from the coarse particulate sample solids. The filtrate is collected in a clean container or graduated cylinder.

1. One fluted filter was carefully opened and inserted into a clean container. (Funnel was used to hold the filter).
2. The edges of the filter were folded over the rim of the cup to hold it in place. Fluted folds of the filter paper were maintained to maximize surface area. This increased speed of the filtration.

3.8 Microfiber Filter

The second filtration step was the gravity filtration of the extract through a microfiber filter. This removed any precipitates in the extract and assured that the extract was easily passed through the affinity column. Microfiber filtration was just performed prior to affinity chromatography.

1. A small funnel was placed in top outlet of the syringe barrel or collecting cup.
2. One microfiber filter was placed gently into the small funnel by pressing filter into funnel with index finger (fingers cleaned with 70% alcohol before and after each sample). Care was taken not to rip or puncture the filter

3.9 Between assays:

After each assay, the blender jar assembly was washed with a mild detergent solution and rinsed thoroughly with water. (The same cleaning procedure was performed for any equipment that was reused to hold, collect or transfer sample extracts). Repipettor was not wash with soap. Methanol repipettor was only refilled with methanol. In-between each assay, the syringe barrel reservoir was rinsed with methanol followed by deionized water. This was sufficient to prevent cross-contamination of samples. After a number of samples have been tested, the glass syringe barrel was washed with a brush and detergent and rinsed well with water.

3.10. Preparation of extraction solutions

The AflaTest procedure uses a methanol or a methanol/water solution to extract aflatoxin out of the sample. To prepare extraction solution: Reagent methanol was used.

TABLE: PREPARATION OF EXTRACTION SOLUTION

SOLUTION DESIRED (methanol; water)	METHANOL (mL)	DISTILED WATER (mL)	TOTAL VOLUME (mL)
80:20	800	200	1000 (1 liter)
70:30	700	300	1000 (1 liter)
60:40	600	400	1000 (1 liter)

3.11 CLEANING EQUIPMENT

3.12 Before Starting AflaTest Testing

To eliminate background fluorescence, it was ensured that the equipment was clean and not contaminated with materials that might cause background fluorescence. This was particularly important when using brand new equipment or equipment that has not been used for a long period of time. Before using the equipment, they were washed with a mild detergent solution and then rinsed thoroughly with purified water. These included the glass syringe barrels used for sample reservoirs. Wash new syringe barrel for pump stands using a brush with soap and water. Then rinse with purified water and methanol. Other pieces of equipment that need to be cleaned with detergent before using are graduated cylinders, funnels and blender jars. Repipettors were only rinsed with methanol before use.

3.13 AFLATEST FLUOROMETER PROCEDURE FOR PEANUT BUTTER

Readout was in parts per billion of total aflatoxin.

3.13.1 Sample Extraction:

1. Twenty-five grams (25g) of ground groundnut paste sample were weighed with 5g NaCl (non-iodized salt) and place in blender jar.
2. 125 mL of methanol: water (70:30) was added to the jar
3. The blender jar was covered and blended at high speed for 2 minutes.
- 4 The cover of the jar was removed to pour extract into fluted filter paper. Filtrate was collected in a clean vessel.

3.13.2 Extraction Dilution

1. Fifteen (15) mL of filtered extract was pipetted into a clean vessel.
2. Extract was diluted with 30 mL purified water and mixed well.
3. The diluted extract was filtered through 1.5µm glass microfiber filter into a clean vessel.

3.13.3 Column Chromatography

1. Fifteen (15) mL (15 mL = 1.0 g sample equivalent) diluted extract completely through AflaTest column at a rate of 1-2 drops/second until air comes through the column.
2. 10 mL of purified water was passed through the column at a rate of 1-2 drops/second.
3. The previous step was repeated once more until air comes through the column.
4. A glass cuvette was placed under the column and 1.0 mL HPLC grade methanol was added into the glass syringe barrel.
5. Columns were eluted at a rate of 1-2 drops/second by passing the methanol through them and collecting all of the sample eluate (1.0 mL) in a glass cuvette
6. 1.0 mL of AflaTest Developer was added to eluate in the cuvette, it was mixed well in the cuvette and placed in a calibrated fluorometer. (VICAM)

Aflatoxin concentration was read after 60seconds

3a



3a

3b



3b

Fig 3a and 3b: Groundnut paste samples used for laboratory analysis

4.0 ANALYSIS OF RESULTS

Ten samples were obtained from the Navrongo community market for the laboratory analysis, each of the samples were run twice to determine the aflatoxin concentrations in them. Sample A was tagged as Test A₁ and A₂, Sample B was tagged as Test B₁ and B₂, C was tagged as Test C₁ and C₂, Sample D was labelled D₁ and D₂, E was labelled E₁ and E₂. The labelling continued to the last sample J and it was labelled Sample J₁ and J₂. We observed that for a particular sample of the groundnut paste there were slight differences in the aflatoxin concentration. The table below shows the AflaTest conducted on the groundnut paste samples and the results we obtained.

Table 4.0 Aflatoxin B1 concentrations in the groundnut paste samples as shown on VICAM

NAME OF TEST	SAMPLE	RESULTS OF TESTS (in parts per billion)		Mean aflatoxin concentration (Parts per billion)
		TEST 1	TEST 2	
AflaTest	A	23 ppb	23 ppb	23ppb
AflaTest	B	45 ppb	45 ppb	45ppb
AflaTest	C	12 ppb	12.2 ppb	12.1ppb
AflaTest	D	8.8 ppb	8.9 ppb	8.85ppb
AflaTest	E	25 ppb	25 ppb	25ppb
AflaTest	F	27 ppb	26.5 ppb	26.75ppb
AflaTest	G	10.8 ppb	10.8 ppb	10.8ppb
AflaTest	H	8.9 ppb	8.9 ppb	8.9ppb
AflaTest	I	24 ppb	22.1 ppb	23.05ppb
AflaTest	J	11ppb	13 ppb	12ppb

*Acceptable level of aflatoxin B1 concentration in groundnut paste is ≤ 20 ppb (less than or equal to 20 parts per billion) ICMSF (Reff:-Codex Standard No.200) year of adoption 1995.

The mean Aflatoxin concentration in each sample was determined to know the actual amount of Aflatoxin B1 concentration in the samples. The possible variations in the results was due to; contaminating a sample whiles working on it, minimal errors in preparation of working solutions as well as preparation of samples, The averages of the test results for each test were calculated and were found to show a slight or no differences in the two tests that were conducted.

AFLATOXIN CONCENTRATIONS IN THE GROUNDNUT PASTES SAMPLES

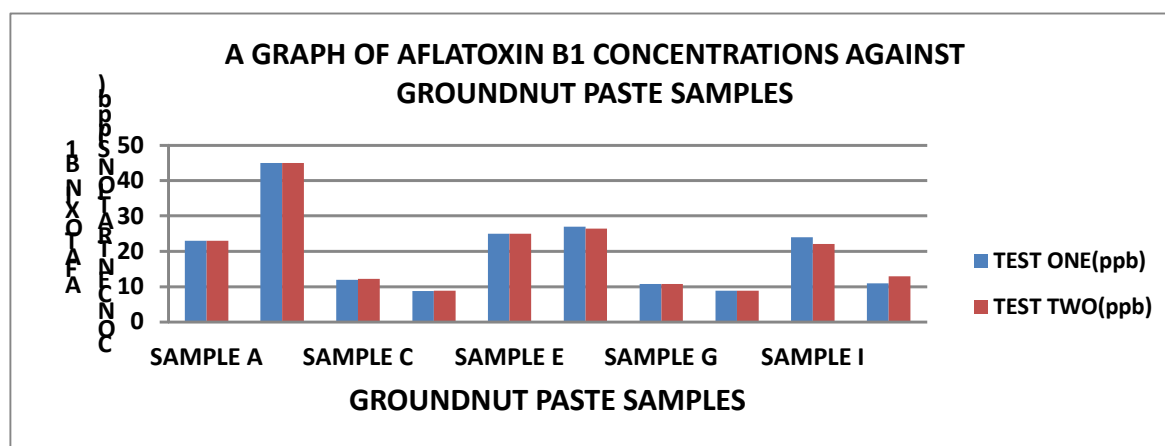


Figure 4: A graph of Aflatoxin concentrations in the groundnut paste samples

It could be observed that out of the ten samples presented to the laboratory for analysis, five samples were showing aflatoxin B1 concentrations above the accepted levels (thus less or equal to 20ppb in Africa). The aflatoxin B1 level in sample B for the two test gave the same concentrations of forty five (45) parts per billion, an indication which shows an abnormal concentration in the groundnut paste sample. Upon careful analysis it was found out that the groundnut paste sample was contaminated before it was collected from the market place. Also samples A, E, F, and I showed aflatoxin B1 levels of (23ppb), (25ppb), (26.75ppb) (23.05ppb) respectively. These range of values, greater than the accepted levels of aflatoxin B1 in the groundnut hence can pose health problems to human beings who consumes them.

5.0 DISCUSSION OF RESULTS

Samples obtained from the probes were grinded and mixed well and a subsample taken for testing. Methanol was made to react with the groundnut paste samples (containing Aflatoxin B1), it dissolved the aflatoxin content in it

and when it was passed through columns which contain antigens, the antibody/antigen binding sites binds the toxins for it to be recorded in the VICAM.

The characterisation of aflatoxin B1 was known based on the smell and its solubility in methanol. The sample, after preparation (thus mixing with 70% methanol and blending) gave a muddy smell which is a clear manifestation of aflatoxin B1. Aflatoxin is also soluble in methanol; the methanol dissolves the aflatoxin B1 present in the sample in order for it to be trapped on antibody/antigen binding surfaces in the columns during the process of affinity chromatography.

According to the food and drug administration of the USA the action level of aflatoxin B1 in peanut paste in Africa is 20 parts per billion (20ug/ml), if all things being equal, any value above 20ppb (20ug/ml) is considered above the action level hence an exposure level of interest. This means that the samples A, B, E, F and I had high aflatoxin level in reference to the limit of 20ppb. (20ug/ml)

Evidence from the various tests done suggested that there was presence of aflatoxins-mainly B1 in the ten samples that were tested. The average aflatoxin levels of samples C (12.1ppb), D (8.85ppb), G (10.8ppb), H (8.9ppb) and J (12ppb) as obtained from the laboratory analysis is an indication that they are in low concentrations below the accepted standard (≤ 20 ppb) hence it does not pose any health danger to consumers.

The variability in the values we obtained is as a result of the origin and places where the groundnut pastes were produced as well as the various conditions under which they were prepared. The variety of groundnut/peanut that was used to prepare the groundnut paste (peanut paste) was also taught to be a contributing factor to the differences in aflatoxin B1 concentrations in the samples.

Chronic exposure to aflatoxins affects human in a number of ways; Stunting and kwashiorkor, which interferes with the metabolism of micronutrients in children, Liver cancer, especially in people with hepatitis B or C as well as Liver diseases.

6.0 CONCLUSION AND RECOMMENDATIONS

In conclusion, the research validity confirmed that,

- Taste of groundnut pastes is not affected when contaminated by aflatoxin B1
- Groundnut paste sold in Navrongo market contains some levels of mycotoxins (aflatoxins.)
- Out of the ten samples that were picked for laboratory analysis, it was revealed that five showed appreciable levels of aflatoxins B1, a little bit higher than the accepted 20ppb standard level of Africa and the Sub Saharan regions; This can pose health problems. The other five samples showed lower levels of aflatoxin B1 concentrations which is not harmful to human health when consumed. ICMSF Codex Standard 200-1995.
- One sample from a well noted part in the Navrongo market contains a very higher level of aflatoxin B1 (45ppb) which could be harmful to consumers.

Based on the findings of our research, we recommend that:

- Pre-harvest, harvesting and post harvest practices of the groundnut should be monitored very well in order to reduce the transfer of contamination to the groundnut paste
- Storing of groundnuts must be in conditions that prevent growth of *Aspergillus* fungi, such as cool air temperatures and low humidity.
- Groundnut paste should be stored in clean dry containers, at a relative humidity below 70% and temperatures between 0 and 10^oC.
- Roasting ,Milling and preparation of groundnut for groundnut paste must be done in a more hygienic environment to reduce the growth of fungi
- Groundnut paste should be stored with enough oil on the surface; this prevents fungus growth which may later lead to aflatoxin infection
- All loosed –shelled ,damaged , and undesired kennels should be examined physically for possible presence of moulds before milling to prevent contamination of the paste
- Defective (mouldy, discolored nut, decayed, insects or otherwise damaged) kennels should be separated from the unaffected ones before milling to produce peanut paste.

Economic impact of mycotoxins

In addition to their direct effects on human health, aflatoxin B1 has a tremendous impact on agriculture. Through spoilage, field crops are rendered useless for animal or human consumption Poor weight gain and outright illness occur in livestock that consume contaminated feeds. The presence of trace quantities in meat, dairy products, and eggs constitutes a further, if largely unconfirmed, health hazard to people.

REFERENCES

1. **Atasie, V.N., Akinhanmi, T.F., Ojiodu, C.C.,** 2009. Proximate analysis and physio-chemical properties of groundnut (*Arachis hypogaea* L.). Pakistan Journal of Nutrition 8 (2), 194-197.

2. **Ames IA**, 2003. Council for Agriculture Science and Technology, "Mycotoxins: Risk in Plants, Animal, and Human Systems" Task force Report 139
3. **Bankole, S., Schollenberger, M., Drochner, W.**, 2006. Mycotoxins in food systems in Sub-Saharan Africa: a review. *Mycotoxin Research* 22 (3), 163-169.
4. **Betina, V.**, 1984. Mycotoxin production, isolation, separation and purification .Elsevier scientific publishers, Amsterdam
5. **Council for Agriculture Science and Technology**. "Mycotoxins: Risks in Plant, Animal, and Human Systems." Task Force Report 139, Ames, IA. 2003
6. **C. W. Heseltine**, 1974 natural occurrence of mycotoxins in cereals
7. **Ciegler, A., S. Kadis & S. J. Ajl** (eds.) (1971) fungal toxins. In 'Microbial Toxins', VOL, 563 pp. Academic Press, New York.
8. **Dorner, J.W.**, 2008. Management and prevention of mycotoxins in peanuts. *Food Additives and Contaminants* 25 (2), 203-208.
9. **Eatan.L and Gallagher, E.P.** (1994) Mechanism of aflatoxin carcinogenesis, *Ann. Rev. Pharmacol. Toxicol* pgs.34, 135-172,
10. **E.Afriyie-Gyau, Kafui A. Kpodo**, (2005) Reducing impact of Mycotoxins in tropical Africa. Food Research institute, Council For Scientific And Industrial Research (CSIR) Ghana
11. **Edinformatics**, 2005.http://www.edinformatics.com/culinaryarts/food_encyclopedia/peanuts.htm. Accessed on 29/11/2005
12. **Egmond, H.P.** 2000. Worldwide regulations for mycotoxins. In DeVries, **J.W.**, **Truckess, M.W.**, **Jackson, L.S.** (Eds) Mycotoxins and food safety. Kluwer Academic Publishers, Norwell. Pp 257-269.
13. **Fink-Gremmels J.**, 1999 Mycotoxins: their implications for human and animal health. *Vet. Q* 21, 115-120.
14. **ICRISAT**, 2005. On-farm management of aflatoxin contamination of groundnut in West Africa. CFC supported Groundnut Seed Project (GSP).
15. **Jones, R.K., Duncan, H.E.**, 1981. Effect of nitrogen fertilizer, planting date and harvest date on aflatoxin production in corn inoculated with *Aspergillus flavus*. *Plant Disease* 65,741-744
16. **Kasno, A.**, 2004. Prevention of *Aspergillus flavus* infection and aflatoxin contamination in groundnut. *Journal Penelitian dan Pengembangan Pertanian* 23 (3), 75-81
17. **Keshri G. & Magan N.**, 2000 Detection and differentiation between mycotoxigenic and non mycotoxigenic strains of two *Fusarium* spp. using volatile production profiles and hydrolytic enzymes. *J. Appl. Microbiol.*, 89(5), 825-833
18. **Magan N. & Evans P.**, 2000. Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. *J. Stored Prod.*, 36, 319-340
19. **Marasas, W.F.O., Gelderblom, W.C.A., Shephard, G.S., Vismer, H.F.**, 2008. Mycotoxins: A global problem. (In Leslie, J.F., Bandyopadhyay, R., Visconti, A., (eds), Mycotoxins: Detection methods, management, public health and agricultural trade. CAB International 2008. Pp. 30.
20. **Mehan, V.K., McDonald, D., Haravu, L.J., Jayanthi, S.**, 1991. The groundnut aflatoxin problem: review and literature database. Patancheru, India. International Crops Research Institute for the Semi Arid Tropics. Pp 1
21. **Mintah, Hunter R**, (1978) The Incidence of Aflatoxin Found in Groundnuts (*Arachis hypogea* L.) Purchased From Markets in and Around Accra, Ghana¹. *Peanut Science: January 1978, Vol. 5, No. 1*, pp. 13-16
22. **Mitullah, W.**, 2003. Street trade in Kenya: the contribution of research in policy dialogue and response. Paper prepared for presentation at the Urban Research Symposium on Urban Development for Economic Growth and Poverty Reduction,
23. **MOFA, U/E** 2012 Data on peanut/groundnut varieties in the Upper East (Navrongo)
24. **Pitt J.I. and Hocking A.D.** Fungi and food spoilage. 2nd ed. Blackie Academic and Professional, London (1997)
25. **Pitt J.I** (2000). Toxigenic fungi and mycotoxins, *Br. Med. Bull.* pgs. 56,184-192
26. **Raney, K.D, Shimada, T et al.**, (1992) Oxidation of aflatoxin and sterigmatocystin by human liver microcosms; Significance of aflatoxin B₁ pgs. 5,251-210
27. **Reddy, T.Y., Sulochanamma, B.N., Subramanyam, A., Balaguravaiah, D.**, 2003. Influence of weather, dry spells and management practices on aflatoxin contamination in groundnut. 56 (3), 262-265.
28. **Richard B Philip**, 2001 human health: toxicology and environmental hazard
29. **Scott, D. B.** (1965). Toxigenic fungi isolated from cereal and legume products. *Mycopathol. Mycol. Appl.* 25: 213-222.
30. **Smith G.** (1960). An introduction to industrial mycology 5th Ed. Edward Arnold Publishers London pp

- 399.
31. **Talawar, S., Rhodes, R.E., Nazarea, V.**, 2005. World Geography of groundnut: Distribution, Use and Trade. <http://lanra.anthro.uga.edu/peanut/publications/wgg.cfm>. Accessed on 2/12/2005.
 32. **Waliyar, F., Natre, B.R., Traore, A., Diarra, B., Kodio, O., Kumar, P.L.**, 2005. Pre- and postharvest management of aflatoxin contamination in groundnut. International Crops Research Institute for the Semi Arid Tropics: Institut d'Economie Rurale (IER), Mali.
 33. **Wu, F.**, 2004. Mycotoxin risk assessment for the purpose of setting international regulatory standards. *Environmental Science and Technology* 38 (15), 4049-4055.
 34. **WHO**, (200) Patulin, in Evaluation of certain food Additives and contaminants. 35th report of the joint FAO/WHO Expert committee on food Additives WHO Geneva pgs.29-30S.