

Distribution of Aflatoxins and Micro Organisms in Peanut and Sunflower Seed Products and their Potential Health Hazards

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Abstract

Background: Aflatoxins are mould metabolites of *Aspergillus flavus* and *parasiticus* that contaminate foodstuffs stored in warm moist places. These toxins are hepatotoxic and produce hepatocellular carcinoma in animals and man.

Objectives: To determine the presence of aflatoxins and microorganisms in ready to consume products of peanuts and sunflower seeds of an edible oil processing factory.

Materials and Methods: Five hundred grams each of peanut kernels, peanut pellets, sunflower seed pellets, peanut oil and sunflower seed oil produced by a local oil extracting factory were supplied for extraction and estimation of aflatoxins and microorganisms like *Salmonella*, *Escherichia coli* (*E.coli*), *Bacillus* and *Moulds*. Aflatoxins B₁, B₂, G₁ and G₂ were extracted in chloroform and concentration was measured using silica gel columns. The quantitative estimation of these aflatoxins B₁, B₂, G₁ and G₂ was done by fluorescence evaluation on Thin Layer Chromatograms.

Results: All test samples contained four types of aflatoxins. The highest aflatoxin values were seen in the pellets and lowest in refined oils. All samples contained higher content of aflatoxins B₁ and B₂ than G₁ and G₂. Refined peanut oil contained much lower aflatoxin than the sunflower seed oil. This study has also identified the presence of several types of microorganisms such as *Salmonella*, *E. coli*, *Bacillus* and *Moulds* in ready to eat peanut and sunflower seed products.

Conclusion: Detection of high levels of aflatoxins and microorganisms in ready to eat food stuff should be a concern for food regulatory agencies since these products are consumed daily by the common man and their cumulative effect on the body might be adding to liver cancer in the population.

Policy message: Food regulatory agencies should regularly carry out thorough surveillance on food stuff.

Key words: Aflatoxins, microbial counts, peanut and sunflower seed products, health hazards.

Introduction

Aflatoxins are a group of highly oxygenated mycotoxins produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus* which contaminate food crops such as cereal grains, oil seeds, and peanuts^{1,2}. Contamination of these commodities occurs in areas where food is produced or stored and where conditions are favorable to mould spoilage and the consequent mycotoxin production³. Hot and humid temperature favors the growth of these moulds⁴. Common toxins that contaminate foodstuffs include Aflatoxins B₁, B₂, G₁, G₂, M₁ and M₂². Among these aflatoxin B₁ is the most toxic

and carcinogenic in both animals and humans affecting primarily liver⁵.

Aflatoxins have been implicated in causing liver cancer in Africa and elsewhere, and the prevalence of hepatocellular carcinoma has been correlated to the extent of aflatoxin contamination in foodstuffs^{6,7}. Human exposure to these toxins can be direct or indirect from food crops and animal products such as smoked meats^{8,9}. Contamination often occurs at pre-harvest, during harvest, and post harvest period when climatic conditions favor the growth of aflatoxin producing fungi².

The biochemical basis of aflatoxin induced cellular damage and carcinogenicity is thought to be through epoxides activating cytochrome P₄₅₀¹⁰⁻¹². The epoxides are trapped as aflatoxin DNA-adducts, and they constitute a critical step in tumor initiation¹¹. Lipid peroxidation and formation of aflatoxin DNA-adducts are the principal manifestations of aflatoxin-induced liver

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cell damage^{5,12}. Moreover aflatoxins have been shown to cause liver cancer in humans⁷ and in laboratory animals¹³.

Because of the potential health hazards associated with the consumption of aflatoxin contaminated food items, this study was carried out to determine the distribution of aflatoxins and microorganisms in edible oil products.

Materials and Methods

The study was carried out at the laboratories of Medical Biochemistry of Moi University School of Medicine and Webuye District Hospital in Kenya. Samples of 500gm each of peanut kernels, peanut pellets, sunflower seed pellets, peanut oil and sunflower-seed oil were supplied by a local Oil Extracting Factory for extraction and estimation of Aflatoxins. About 100gm of peanut kernel was first pounded in a porcelain mortar and then blended in a high speed blender. For each peanut and sunflower-seed pellet, thorough mortar-pounding was done before blenderizing it. Subsequent extraction and purification of aflatoxins was carried out by a slightly modified version of method of Epply¹⁴ for peanut and oil containing products. This modification included extraction in chloroform, concentration on silica gel columns and quantitative estimation of aflatoxins B₁, B₂, G₁ and G₂ by fluorescence evaluation on thin-layer chromatograms (TLC). The sensitivity of this method is equal to 1µg/kg (1ppb). The assay results were coded onto IBM cards for statistical analysis with the help of Moi University Computer Centre. The Aflatoxin concentration (µg/kg) for peanut and sunflower seed products were analyzed by Microsoft Excel Software 2003, using aflatoxin standards containing 10µg/ml (Table-1).

Table 1: Quantitative estimation of aflatoxins in peanut and sunflower-seed products.

Sample	N	Aflatoxin Content per 100g Sample			
		AFB ₁	AFB ₂	AFG ₁	AFG ₂
Peanut kernels	100g	524±3.5	376±3.8	144±2.6	103±1.5
Peanut pellets	100g	840±3.0	650±3.4	184±2.4	105±1.5
Sunflower seed pellets	100g	740±3.2	510±3.5	164±2.5	110±1.2
Peanut oil	100g	12±0.6	10±0.6	10±0.6	12±0.6
Sunflower seed oil	100g	24±0.2	18±0.4	20±0.4	18±0.4

Aflatoxin concentrations are expressed as means ± S.E. in µg/kg: ppb
N is the number in grams of the test samples used.

Total and differential microbial counts on peanut kernels, peanut pellets, and sunflower seed pellets were carried out using a slightly modified method of Abalaka and Elegbede³ where microbial counts were carried out on 300 gm samples of peanut kernels, peanut pellets and

sunflower seed pellets. For all microbial counts 100ml peptone water was added to 100gm sample and the mixture was incubated for 8hrs with occasional shaking. A serial dilution (1/10) of all original samples were carried out in peptone water after every 5-minute to avoid microbial cell injury. 0.1ml sample from each serial dilution was then transferred into three plates. The plates were swirled and incubated at intervals of 25^oC, 30^oC, and 37^oC for 24hr at each interval. The shifting period was to promote maximum microbial growth. Colonies growing on the plates were counted after 72hr using a colony counter and recorded (Table-2).

Table 2: Microbial counts on samples of peanut and sunflower seed pellets.

Samples	N (g)	Number of Microbial Count for 100g Sample				
		Bacillus	Moulds ⁺	E. Coli	Salmonella	Total
Peanut kernels	100	412	143	20	13	4x10 ⁷
Peanut pellets	100	20	11	5	3	5x10 ³
Sunflower seed pellets	100	13	21	4	2	4x10 ²

* Counts are means of three counts on each of three plates used without pre-enrichment.

+ Moulds were counted as number of colony-forming unit per 1090g sample.

N is the number in grams of the test sample used

Results

All samples of peanut and sunflower seed products namely, peanut kernels, peanut pellets, sunflower seed pellets, peanut oil and sunflower seed oil contained substantial amounts of Aflatoxins B₁, B₂, G₁ and G₂ (Table-1). Highest aflatoxin values were recorded in kernels and pellets while, the lowest values were recorded in refined oil products. Both the pellets and the kernels contained high values of aflatoxins B₁ and B₂ than G₁ and G₂. Mean values for B₁ and B₂ were 524µg/kg and 376µg/kg for kernels respectively. For peanut pellets, mean values for B₁ and B₂ were 840µg/kg and 650µg/kg respectively. For sunflower seed pellets, mean values for B₁ and B₂ were 740µg/kg and 510µg/kg respectively. Mean values for peanut oil ranged from 10 to 12µg/kg for all Aflatoxins (B₁, B₂, G₁ G₂) while, for sunflower seed oil the range was between 18 to 24µg/kg for all Aflatoxins.

A total of 300gm samples of peanut kernels, peanut pellets and sunflower-seed pellets were examined for microbial counts and types of micro organisms. The results of these determinations are shown in Table-2. The kernels contained highest number of Bacillus (412 counts) and moulds (143 counts) while, peanut pellets contained

lowest number of *Salmonella* (3 counts) and *E.coli* (5 counts). In sunflower-seed pellets, the lowest microorganism numbers recorded were *Salmonella* (2 counts), followed by *E.coli* (4 counts), *Bacillus* (13 counts) and moulds (21 counts) (Table-2). Generally, pellets had low bacterial counts as compared to those for kernels.

Discussion

This study revealed that the peanut and sunflower seed products derived from an edible oil processing factory were contaminated with aflatoxins and micro organisms. The levels of aflatoxin contamination were sufficient to cause major concern to food regulatory agencies and to consumers.

Aflatoxin contamination can occur at any stage from pre-harvest to harvest and post harvest period^{2,6}. Since these products were purchased by the factory directly from the farmers and stored in the factory's premises before processing therefore, contamination probably must have occurred in the factory's storage facility contrary to the views of other similar investigators³ as they have not been able to establish the origin of the contamination³.

Several factors could be responsible for the observed higher levels of aflatoxins in the pellets than in the kernels. It is possible that peanut kernels may have been relatively free of contamination when delivered from the farmers but storage conditions may have caused rapid mould growth and consequent aflatoxin production¹⁵. Other possibility is that since pellets represent concentrated form of solid part of nuts and seeds, therefore, a greater amount of aflatoxins would be pressed into the pellets than then that found in the liquid containing residue after processing. Similar observations have been made by other investigators^{3,6}.

Presence of substantial amounts of aflatoxins in peanut and sunflower seed oil proves that aflatoxin contamination did not occur on unpelleted products supplied by the farmers. Since these oils are consumed almost on daily basis in many parts of the world, therefore, presence of aflatoxins in them could have a cumulative effect on the health of consumers. The hepatocarcinogenic and hepatotoxic effect of aflatoxin consumption has been reported by other worker¹⁶.

Presence of micro-organisms in peanut and sunflower seed products was not unexpected. Temperature and humidity, enhancing aflatoxin production could also have enhanced the growth and proliferation of micro-organisms on stored peanuts and sunflower seeds causing disease in animals and human beings as these micro organisms are known to produce health problems¹⁷.

Aflatoxins are cancer causing agents¹⁶, and they are easily transmitted to the eggs when consumed by egg laying hens¹⁸, therefore, their presence in peanut and

sunflower seed pellets should be viewed with considerable concern. This is because several feed processing companies use these pellets as raw material for producing a variety of edible oils and feed for egg laying chickens. Since these oils and chicken eggs are consumed daily, they could have a cumulative carcinogenic effect on human liver^{7,19}.

The present study showed the presence of aflatoxins and micro organisms in the products of an edible oil processing factory indicating poor storage in the factory. Since consumers have a high chance of consuming aflatoxin contaminated chicken eggs and edible-oils, there is an urgent need to improve the storage and processing of these food products. The food regulatory agencies also need to be more vigilant through regular checking and surveillance to avoid these health hazards.

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